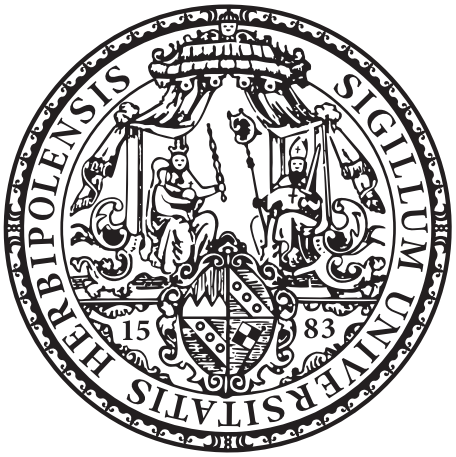

Electrocortical mechanisms of sustained attention during the acquisition and interaction of conditioned fear and anxiety

Elektrokortikale Mechanismen der Aufmerksamkeit während der Akquisition und Interaktion konditionierter Furcht und Angst

Doctoral thesis for a doctoral degree

at the Graduate School of Life Sciences,

Julius-Maximilians-Universität Würzburg,

Section Neuroscience

submitted by

Yannik Stegmann

from

Aschaffenburg

Würzburg 2021

Submitted on:

Members of the Thesis Committee:

Chairperson: Prof. Dr. Carmen Villmann

Primary Supervisor: Prof. Dr. Paul Pauli

Supervisor (Second): Prof. Dr. Matthias J. Wieser

Supervisor (Third): Prof. Dr. Erhard Wischmeyer

Supervisor (Fourth): Prof. Dr. Matthias Gamer

Date of Public Defense:

Date of Receipt of Certificates:

Acknowledgements

Mit dem Abschluss dieser Arbeit endet nicht nur meine Zeit als Doktorand, sondern auch ein weiterer Lebensabschnitt. Die vorliegende Arbeit stellt dabei lediglich das Endprodukt der letzten vier Jahre dar und repräsentiert sie vermutlich ähnlich oberflächlich wie das zweidimensionale Abbild eines dreidimensionalen Körpers. Ein wichtiger Teil, der beim Schreiben und Lesen dieser Arbeit verloren geht, ist die Anzahl der Personen, die mich über diesen Zeitraum begleitet und dabei wesentlich zur Entstehung meiner Dissertation beigetragen haben. Ich bin sehr glücklich darüber, in den letzten Jahren so vielen brillianten Wissenschaftlern und einzigartigen Menschen begegnet zu sein.

An erster Stelle danke ich meinen Betreuern, Prof. Dr. Paul Pauli und Prof. Dr. Matthias J. Wieser für die Unterstützung in den letzten vier Jahren, den fachlichen Austausch, die inhaltlichen Diskussionen und dafür, dass Sie mich mit dem Thema der Furcht- und Angststörungen in einem biopsychologischen Kontext vertraut gemacht haben. Ich möchte mich vor allem auch für die Förderung bedanken, die ich erhalten habe und die mich tiefer in das Thema hat einsteigen lassen. Vielen Dank auch für die tolle Betreuung trotz den mehrere hundert Kilometer zwischen Würzburg und Rotterdam. Des Weiteren möchte ich mich bei Prof. Dr. Matthias Gamer und Prof. Dr. Erhard Wischmeyer als Teil meines Promotionskomitees für die tollen Diskussionen, Vorschläge und Unterstützung bei der Entwicklung meines Promotionsthemas bedanken.

Einem besonderen Dank gebührt auch Philipp Reicherts, der mich zusammen mit Matthias Wieser sogar schon vor meiner Promotion im Rahmen meiner Masterthesis betreut hat. Darüber hinaus war Philipp immer mein erster Ansprechpartner, wenn es um die Planung und Diskussion der Details oder um die Auswertung der ersten Daten einer Studie geht. Vielen Dank auch dafür, dass du immer ein offenes Ohr hattest und mich vor allem beim Einstieg in die Wissenschaft so sehr unterstützt hast! Vielen Dank auch an Christopher Klinke, der mich nicht nur im Co-Projekt, sondern auch auf sämtliche

Konferenzen begleitet hat. Danke für die intensiven Diskussionen, die gemeinsame Zeit in und außerhalb des Büros und die vielen echt lustige Witze. Danke für deine Unterstützung und Freundschaft!

*Einen großen Dank auch an meine Bürokolleginnen, Octávia Madeira und Katharina Herzog, für die spannenden und lehrreichen Diskussionen sowie die lustigen Momente im Büro. Außerdem möchte ich mich bei Hannah Genheimer und Marta Andreatta bedanken, die zusammen mit Christopher und Katharina, in unseren Konditionierungs-Meetings für tollen inhaltlichen Austausch sorgten. Weiter danke ich Daniel Gromer, Lea Hildebrandt, Jonas Großekathöfer und Mario Reutter für die aufschlussreichen methodischen Diskussion und für das Einführen in die Welt der Open Science. Genauso danke ich allen anderen Kolleg*innen des Lehrstuhls für Psychologie I für die Unterstützung und die tolle gemeinsame Zeit. Außerdem danke ich Andreas Keil für die Gastfreundschaft in Gainesville und dafür dass du mir ein noch tieferes Verständnis von ssVEPs und Neurowissenschaften im Allgemeinen ermöglicht hast. In diesem Zusammenhang möchte ich mich auch bei Maeve Boylan für die Antworten auf die unzähligen Fragen bedanken und dafür, dass du sichergestellt hast, dass mein Englisch annähernd vorzeigbar ist.*

Darüber hinaus gibt es eine Reihe an wundervollen Menschen, die mich hinter den Kulissen durch die letzten Jahre begleitet und unterstützt haben. Dazu zählt in erster Linie meine Familie; meine Eltern, die mir das Studium überhaupt erst ermöglicht haben und mein Bruder, mit dem ich in den ersten Semestern in Würzburg sogar eine WG teilte. Vielen Dank für eure jahrelange Unterstützung und für euer Verständnis für die Zeit, die ich kürzlich nicht mit euch verbringen konnte. Und schließlich gilt meinen tiefsten Dank meiner Freundin, die immer für mich da war und mich bei allem unterstützt hat. Danke, dass du mich in den letzten Jahren begleitet hast und mir den notwendigen Ausgleich geschaffen hast.

Table of Contents

List of Tables

List of Figures

Abstract

Adapting defensive behavior to the characteristics of a threatening situation is a fundamental function of the brain. Particularly, threat imminence plays a major role for the organization of defensive responses. Acute threat prompts phasic physiological responses, which are usually associated with an intense feeling of fear. In contrast, diffuse and potentially threatening situations elicit a sustained state of anxious apprehension. Detection of the threatening stimulus defines the key event in this framework, initiating the transition from potential to acute threat. Consequently, attention to threat is crucial for supporting defensive behavior. The functions of attention are finely tuned to the characteristics of a threatening situation. Potential threat is associated with hypervigilance, in order to facilitate threat detection. Once a threatening stimulus has been identified, attention is selectively focused on the source of danger. Even though the concepts of selective attention and hypervigilance to threat are well established, evidence for their neural correlates remain scarce. Therefore, a major goal of this thesis is to elucidate the neural correlates of selective attention to acute threat and hypervigilance during potential threat. A second aim of this thesis is to provide a mechanistic account for the interaction of fear and anxiety. While contemporary models view fear and anxiety as mutually exclusive, recent findings for the neural networks of fear and anxiety suggest potential interactions. In four studies, aversive cue conditioning was used to induce acute threat, while context conditioning served as a laboratory model of potential threat. To quantify neural correlates of selective attention and hypervigilance, steady-state visual evoked potentials (ssVEPs) were measured as an index of visuocortical responding. Study 1 compared visuocortical responses to acute and potential threat for high versus low trait-anxious individuals. All individuals demonstrated enhanced electrocortical responses to the

central cue in the acute threat condition, suggesting evidence for the neural correlate of selective attention. However, only low anxious individuals revealed facilitated processing of the contexts in the potential threat condition, reflecting a neural correlate of hypervigilance. High anxious individuals did not discriminate among contexts. These findings contribute to the notion of aberrational processing of potential threat for high anxious individuals. Study 2 and 3 realized orthogonal combinations of cue and context conditioning to investigate potential interactions of fear and anxiety. In contrast to Study 1 and 2, Study 3 used verbal instructions to induce potentially threatening contexts. Besides ssVEPs, threat ratings and skin conductance responses (SCRs) were recorded as efferent indices of defensive responding. None of these studies found further evidence for the neural correlates of hypervigilance and selective attention. However, results for ratings and SCRs revealed additive effects of fear and anxiety, suggesting that fear and anxiety are not mutually exclusive, but interact linearly to organize and facilitate defensive behavior. Study 4 tested ssVEPs to more ecologically valid forms of context conditioning, using flickering video stimuli of virtual offices to establish context representations. Contrary to expectations, results revealed decreased visuocortical responses during sustained presentations of anxiety compared to neutral contexts. A disruption of ssVEP signals eventually suggests interferences by continuously changing video streams which are enhanced as a function of motivational relevance. In summary, this thesis provided evidence for the neural correlates of attention only for isolated forms of fear and anxiety, but not for their interaction. In contrast, an additive interaction model of fear and anxiety for measures of defensive responding offers a new perspective on the topography of defensive behavior.

Zusammenfassung

Die Anpassung defensiver Verhaltensweisen an die Anforderungen bedrohlicher Situationen ist eine fundamentale Funktion des Gehirns. Akute Bedrohung führt in der Regel zu kurz-anhaltenden, physiologischen Reaktionen, die mit einem Gefühl intensiver Furcht einhergehen, während Situationen potenzieller Bedrohung zu einem anhaltenden Zustand erhöhter Angst führen. Dabei spielt das Erkennen der Gefahr eine besondere Rolle, da sie den Übergang von potenzieller zu akuter Bedrohung initiiert. Demnach kommt der Aufmerksamkeit eine wichtige Funktion bei der Unterstützung defensiver Verhaltensweisen zu. Mechanismen der Aufmerksamkeit sind dabei präzise auf die jeweilige Situation abgestimmt. Potenzielle Bedrohung führt zu Hypervigilanz, um bedrohliche Reize schneller zu entdecken. Sobald eine Bedrohung identifiziert wurde, wird die Aufmerksamkeit selektiv auf diese fokussiert. Obwohl die Konzepte der Hypervigilanz und selektiven Aufmerksamkeit gut etabliert sind, stehen Befunde für ihre neuronalen Korrelate noch aus. Dementsprechend ist ein Ziel dieser Doktorarbeit, die neuronalen Korrelate von Hypervigilanz bei potenzieller Bedrohung und selektiver Aufmerksamkeit auf akute Bedrohung zu erforschen. Ein weiteres Ziel ist es, ein interaktives Modell der Furcht und Angst zu testen. Bisherige Modelle stellen Furcht und Angst als zwei sich gegenseitig ausschließende Zustände dar, allerdings legen Befunde über die neuronalen Netze von Furcht und Angst nahe, dass sie sich gegenseitig beeinflussen könnten. In insgesamt vier Studien wurde aversive Cuekonditionierung zur Induktion akuter Bedrohung genutzt, während Kontextkonditionierung als experimentelles Modell potenzieller Bedrohung diente. Zur Quantifizierung der visuokortikalen Korrelate selektiver Aufmerksamkeit und Hypervigilanz wurden steady-state visuell evozierte Potentiale (ssVEPs) gemessen. Studie 1 verglich visuokortikale Verarbeitung bei akuter und potenzieller Bedrohung zwischen

hoch- und niedrig-ängstlichen Probanden. Im Sinne der selektiven Aufmerksamkeit zeigten alle Probanden eine erhöhte Verarbeitung der visuellen Reize, die akute Bedrohung vorhersagten. Hypervigilanz zeigte sich allerdings nur bei Niedrig-ängstlichen, die Kontextreize, die mit potenzieller Bedrohung assoziiert waren, stärker verarbeitet, während Hoch-ängstliche nicht zwischen den Bedingungen diskriminierten. Dieses Ergebnis spricht für eine dysfunktionale attentionale Verarbeitung potenzieller Bedrohung bei hoch-ängstlichen Individuen. In Studie 2 und 3 wurde eine orthogonale Kombination aus Cue- und Kontextkonditionierung implementiert, die eine Untersuchung der Interaktion zwischen Furcht und Angst ermöglichte. Im Gegensatz zu Studie 1 und 2 wurden in Studie 3 potenziell bedrohliche Kontexte durch verbale Instruktion realisiert. Neben ssVEPs wurden subjektive Angaben der wahrgenommenen Bedrohung und Hautleitfähigkeitsreaktionen (SCRs) als Maße defensiver Verhaltensweisen aufgezeichnet. Studie 2 und 3 zeigten keine Hinweise für die neuronalen Korrelate selektiver Aufmerksamkeit und Hypervigilanz. Allerdings konnten für Ratings und SCRs additive Effekte von Furcht und Angst festgestellt werden. Dieses Ergebnis deutet an, dass sich Furcht und Angst nicht gegenseitig ausschließen, sondern linear interagieren, um defensive Verhaltensweisen zu unterstützen. In Studie 4 wurde ein Kontextkonditionierungsparadigma mit Videos virtueller Büroräume durchgeführt, um visuokortikale Aktivität während ökologisch valideren Kontexten zu testen. Entgegen der Erwartungen zeigten die Ergebnisse eine reduzierte visuokortikale Aktivität während des Angst- im Vergleich mit dem neutralen Kontext. Eine Reduktion der visuokortikalen Aktivität könnte auf Interferenzen durch die kontinuierlich verändernden Videos hindeuten, welche in Abhängigkeit motivationaler Relevanz verstärkt wurden. Zusammengefasst liefert diese Arbeit Hinweise auf neuronale Korrelate der Aufmerksamkeit bei isolierten Formen von Furcht und Angst, nicht aber bei deren Interaktion. Im Gegensatz dazu, bietet das additive Modell der Furcht und Angst eine neue Perspektive auf die neurowissenschaftliche Organisation defensiver Verhaltensweisen.

Theoretical background

Attention to threat is crucial for the organism's chances for survival. The detection of threat usually triggers a cascade of defensive mechanisms observable from a macro level of behavioral output to a micro level of autonomic function, which can be perceived in practically all species, including humans. Therefore, learning to detect threat is a fundamental function of the brain and ensures adaptive behavior to ever changing environments. Given many forms of threat, an organism's survival is dependent on selective responses and thus the efficiency of defensive behavior. However, this task is not as trivial as it might seem. For example, perceiving every novel stimulus as threatening and unnecessarily activating defensive responses would result in an immense waste of vital resources. In contrast, belated responding to an actual threat could be fatal. Consequently, the organism's ability to detect threat needs to ensure the use of optimal defensive strategies, including timely responses to stimuli that are actually capable of inflicting harm. In humans, a disruption of these abilities lies at the heart of many mental disorders, especially of anxiety disorders.

The key characteristic of anxiety disorders is excessive fear and anxiety (American Psychiatric Association, 2013), which - from an external point of view - is often perceived as disproportionate to the actual threat. For example, generalized anxiety disorder is characterized by excessive worrying about events or activities (American Psychiatric Association, 2013), while patients with phobic-related disorders are afraid of specific objects (e.g. spiders or dogs) or situations (e.g. flying or heights). Likewise, patients with social-anxiety disorder fear social interaction. A common feature underlying all major anxiety disorders is that the symptoms are often described as ir-

rational and exaggerated, hinting at dysfunctional threat processing mechanisms. In Europe, the burden of anxiety disorders for society and the public healthcare systems is immense (Wittchen et al., 2011). With a prevalence of 10% (Baxter, Scott, Vos, & Whiteford, 2013), anxiety disorders are considered as one of the most common mental disorders. For that reason, basic and clinical researchers alike grew interested in the investigation of anxiety disorders, particularly in the development, maintenance, and treatment of pathological forms of fear and anxiety.

Over the last century, important milestones have been reached and, with cognitive-behavioral therapy, powerful tools for the treatment of anxiety disorders have been developed (Carpenter et al., 2018). However, even though cognitive-behavioral therapy is considered to be the most efficacious intervention for anxiety disorders (Hofmann & Smits, 2008), a substantial number of patients do not respond to the treatment or may experience a return of fear after therapy (Butler, Chapman, Forman, & Beck, 2006; Loerinc et al., 2015). In addition, the underlying mechanisms of pathological anxiety, especially on neurophysiological level, remain elusive. The unveiling of those mechanisms is an ongoing scientific task and to date little consensus on their involvement in the development and maintenance of pathological anxiety has been reached. More strikingly, prior to addressing the dysregulation of threat processing mechanisms, a precise mechanistic account of those functions in the healthy brain has yet to be discovered. Therefore, it is of utmost importance to promote the fundamental research of threat processing mechanisms for the understanding of healthy and pathological forms of fear and anxiety.

1.1 Threat detection and defensive responding

On a most fundamental level, threat processing functions can be separated into ‘threat detection’ and ‘defensive responding’ (e.g. LeDoux in Mobbs et al., 2019; Fanselow,

2018; LeDoux, 2014), where threat detection describes the ability to recognize and integrate threat cues from the environment (Hamm, 2020), while defensive responding summarizes all responses that are activated by an actual or perceived threat (LeDoux, 2014). Threat detection starts with the sensory processing of inputs from the visual, auditory, and olfactory system, which are integrated in later stages of the processing hierarchy to inform about the presence or absence of an upcoming danger (Silva, Gross, & Gräff, 2016). Defensive responding includes reactions of the endocrine and autonomic nervous systems, as well as species-specific behavioral responses, like the fight, flight, or freeze response in rodents (Bolles, 1970). However, the boundaries between threat detection and defensive responding are not well defined and a lot of research focuses on the mechanisms and circuits, which characterize their interaction, rather than each individual function. To improve our understanding of how organisms respond to threat, it is important to focus on the different functions of threat detection and defensive responding in the cascade of threat processing mechanisms.

In this regard, defensive responding is usually considered as hard-wired, innate behavior, which is not shaped by previous experience (Bolles, 1970). From an evolutionary perspective, the topography of defensive behavior could not be based on trial-and-error learning, since the outcome of a single failure is most certainly lethal for the organism. As a consequence, defensive responding primarily reflects the phylogenetic history of a species, resulting in a hard-wired behavior, which is carried out reflexively once a threat is identified (Bolles, 1970; Fanselow, 2018).

While defensive responding is most likely innate, recognition of threat is not (Fanselow, 2018). On one hand, having the information and response patterns to encode all different kind of threats already genetically stored would be immense and expend a large amount of genetic capacity. On the other hand, if the organism needs to rely on only its evolutionary history, it would not be able to cope with threats occurring in the environment for the first time. Consequently, learning mechanisms play

a major role in the formation of threat detection mechanisms. The most fundamental way the brain learns to recognize novel threats is by Pavlovian fear conditioning.

1.2 Learning mechanisms in threat detection

1.2.1 Pavlovian fear conditioning and extinction learning

Pavlovian fear conditioning is a special variant of classical conditioning (Pavlov, 1927). In this terminology, a novel, neutral stimulus (NS) is repeatedly associated with an unconditioned stimulus (US), which triggers the unconditioned response (UR). After several pairings, the sole presentation of the formerly neutral stimulus, now called the conditioned stimulus (CS), is enough to elicit a conditioned response (CR). In fear conditioning, the CS is usually a discrete visual or auditory cue that predicts an upcoming aversive event (US), like a mildly painful stimulus or a loud, unpleasant noise. Typical readouts of fear are subjective-verbal, physiological, and behavioral responses to the conditioned stimuli (Bradley & Lang, 2000).

In the laboratory, the most common approach to study fear learning in humans is the differential fear conditioning paradigm (Lonsdorf et al., 2017), in which one conditioned stimulus (CS+) is paired with an aversive event (US), while a second conditioned stimulus (CS-) is never associated with the US. Using differential fear conditioning offers greater statistical power as responses to the CS+ are contrasted to the CS- and thereby reduces between-subject variance (Lonsdorf et al., 2017). It is important to note, however, that while the CS+ signals upcoming threat, the CS- is not completely neutral, but indicates the absence of threat and therefore might serve as a safety signal (Lissek et al., 2005; Seligman & Binik, 1977). These considerations must be taken into account, especially with fear conditioning paradigms becoming more complex.

Scientists interested in the stability of fear conditioning frequently include an

additional phase, in which the former CS+ is repeatedly presented without US reinforcement. During this phase, extinction learning takes place and the conditioned responses gradually decline (Graham & Milad, 2011). Extinction learning is assumed to form a new CS-noUS association, which inhibits, rather than erases the original CS-US association (Bouton, 2004; Milad & Quirk, 2012). Therefore, extinction represents an adaptive re-learning mechanism in situations where former threat-signaling stimuli no longer predict aversive events (Milad & Quirk, 2012). Critically, extinction learning is discussed as one of the main mechanisms underlying exposure therapy of pathological fear (Pittig, Berg, & Vervliet, 2016), where prolonged exposure to the phobic object helps to reduce fear. Even though exposure therapy is highly effective, recent treatment studies indicate high numbers of nonresponse and dropout (Pittig et al., 2016; Richter, Pittig, Hollandt, & Lueken, 2017). Consequently, enhancing our understanding of extinction learning should also lead to improvements of exposure therapy (Graham & Milad, 2011).

Pavlovian fear conditioning and extinction learning are often used as basic models of fear-learning mechanisms because they can easily be implemented in controllable laboratory settings and, most importantly, they can be utilized for human and animal research alike and thus benefitting from a cross-species translational perspective (Haaker et al., 2019; Milad & Quirk, 2012; Ojala & Bach, 2020). However, there are also limitations to a cross-species translational perspective with different methodological protocols and divergent theories leading to challenges in the interpretaton of translational research. Hence, it is important to focus on both translational and human models of psychopathology to understand healthy and clinical fear and anxiety (Grillon, Robinson, Cornwell, & Ernst, 2019).

1.2.2 The role of learning theories in anxiety disorders

Regarding clinical anxiety, fear conditioning is the most prevailing model for the development of pathological fears and etiology of anxiety disorders in humans (Mineka & Zinbarg, 2006). Beginning with the famous 'Little Albert experiment', in which Watson & Rayner (1920) demonstrated that an infant acquires a fear of white rats, after they had been associated with a loud, unpleasant noise, there has been tremendous progress in understanding the role of learning theories in anxiety disorders (Mineka & Oehlberg, 2008). In these associative learning models the principles of fear conditioning are directly transfered to the delevopment of anxiety disorders (Pittig, Treanor, LeBeau, & Craske, 2018) and thus, these models yield a high face validity (Vervliet & Raes, 2013). For example, a paramedic learned about a serious accident while driving in the ambulance on a highway (CS), eliciting feelings of panic (US). According to learning thories, this incident will result in the formation of an association between the experience of panic and driving on highways. As a consequence, driving on highways produces distress and fear, even if the paramedic is merely a passenger, potentially giving rise to the development of a panic disorder. Indeed, it could be shown that a disruption of fear conditioning processes predicts the development (Lommen, Engelhard, Sijbrandij, Hout, & Hermans, 2013) and the symptom severity of posttraumatic stress disorder (Sijbrandij, Engelhard, Lommen, Leer, & Baas, 2013). Moreover, meta-analyses on fear conditioning in clinical anxiety revealed that patients with anxiety disorders were characterized by stronger fear responses, decreased differential responding, and slower extinction learning rates compared to healthy individuals (Duits et al., 2015; Lissek et al., 2005).

It is important to mention, however, that there is also criticism about the role of learning mechanisms in anxiety disorders (Beckers, Krypotos, Boddez, Effting, & Kindt, 2013; Vervliet & Boddez, 2020). First, fear conditioning might be a good

model of some anxiety disorders, e.g. panic disorder or specific and social phobias, where the phobic stimulus can easily be identified, but it struggles to explain other types of anxiety disorders, e.g. generalized anxiety disorders, where a distinct learning experience is often missing. Second, many patients with specific phobias never had or are not able to recall any aversive events that were associated with their phobic stimulus (Askew & Field, 2007; Withers & Deane, 1995). Third, learning theories often underestimate the importance of (meta-)cognitive factors underlying anxiety disorders, like the perceived controllability over aversive events or the subjective belief that anxious worrying is harmful (Hofmann, 2008; Wells & Matthews, 1996). For these reasons, it might be insufficient to consider only learning theories in the development and maintenance of anxiety disorders. Still, it is without doubt that dysfunctional learning mechanisms lie at the core of many anxiety disorders and that uncovering the fundamental processes underlying fear conditioning and extinction is a primary goal of the (bio-)psychological research to better understand their contributions and limitations to pathological fear and anxiety.

1.2.3 Mechanisms underlying fear conditioning

There are different theories regarding the underlying mechanisms of fear conditioning. Traditional views see fear conditioning as a variation of associative learning, where stimulus-stimulus associations between the CS and the US are formed and the CS substitutes the US in eliciting the unconditioned response (S-S learning model; Pavlov, 1927). S-S learning models rapidly grew in popularity because they parallel early findings of synaptic plasticity on the neurobiological, cellular level. Hebbs' theory (Hebb, 1949) postulates that neurons that are activated at the same time, form stronger synaptic associations. During fear conditioning, the simultaneous activation of neurons that respond to the CS and neurons that respond to the US should strengthen their synaptic associations and form more densely connected neural net-

works (Blair, Schafe, Bauer, Rodrigues, & LeDoux, n.d.). Thus, Hebbian plasticity provides a neurobiological account for the underlying mechanisms of fear conditioning.

Another advantage of associative learning models is that they can be quantified by computational models. The Rescorla-Wagner Model (Rescorla & Wagner, 1972) assumes that the associative strength between CS and US is driven by previous experience. Learning occurs in the form of updating a prediction error on a trial-by-trial basis. In the beginning of fear conditioning, the associative strength between CS and US is low. The simultaneous presentation of the CS and the US prompts a prediction error, which increases the associative strength between the stimuli for the next trial. By incorporating these concepts into a mathematical formula, learning can be predicted on a single-trial level. Importantly, the Rescorla-Wagner model can also be employed in differential fear conditioning paradigms (Rescorla & Wagner, 1972). Computational models are able to explain many basic conditioning and extinction phenomena and there are various extensions of the Rescorla-Wagner model that account for more complex designs (for a review, see Le Pelley, 2004). It is also important to mention, that participants do not necessarily need to be aware of the CS-US contingencies and that prediction errors shape learning without conscious expectations (Moratti & Keil, 2009). In these models, the associative strength between CS and US can even be completely independent of the cognitive anticipation (Perruchet, 1985; Perruchet & Pacton, 2006; Yuan, Giménez-Fernández, Méndez-Bértolo, & Moratti, 2018).

In contrast, there are theories of fear conditioning claiming that learning cannot occur without conscious awareness (Lovibond & Shanks, 2002). These propositional learning theories assume that associations between stimuli are not shaped automatically but need to rely on higher-cognitive processes to form mental representations (propositions) about the relationship between CS and US. There are many studies

demonstrating the effect of verbal instructions (Mertens, Boddez, Sevenster, Engelhard, & De Houwer, 2018) or observational learning (Haaker, Golkar, Selbing, & Olsson, 2017) that can be better explained with propositional learning theories. However, these theories cannot account for all conditioning phenomena. As the debate between autonomic and propositional learning is still ongoing, a reasonable compromise is provided by the dual-system approach (Mitchell, De Houwer, & Lovibond, 2009). The dual-system approach combines propositional and associative learning and thereby puts forth a flexible model of the psychological mechanisms underlying fear conditioning. However, for a better understanding of the underlying mechanisms, it is also important to consider the neural substrates of fear conditioning.

1.2.4 Neurobiological circuits underlying fear conditioning and extinction learning

Identical to the psychological mechanisms, there is a large body of research focusing on the neurobiological mechanisms of fear learning and fear expression. In line with the translational perspective, many important results stem from animal studies. Converging evidence indicates a neural circuit with the amygdala as a central hub, responsible for the expression and acquisition of fear (Davis, 1992). In more detail, sensory information about the conditioned stimulus enters the basolateral nuclei of the amygdala through pathways from thalamic nuclei and auditory or visual cortices. The basolateral nuclei have direct projections to the central amygdala, which is connected to brainstem areas that control the expression of fear responses. Importantly, the formation of novel associations via fear conditioning could also occur in the amygdala, where pathways transmitting CS and US information converge (LeDoux, 2000). The basolateral amygdala and the central amygdala also receive nociceptive inputs (of the US) from other brain areas, which could be integrated with the sensory information of the CS to induce plasticity in these areas (LeDoux, 2000).

Recently, novel tools like optogenetics and other refined in-vivo imaging techniques greatly advanced our understanding of the neural fear circuits and provided detailed insights into the components and neural connections involved (Tovote, Fadok, & Luthi, 2015). Importantly, the results of these studies mainly substantiated existing networks, but they were also able to identify additional areas like the prefrontal cortex and the hippocampus, which play important roles in the neural fear circuit. In summary, neurobiological research in animals were able to reliably identify a widely distributed neural network underlying fear conditioning. However, translating these results from animal work to humans is challenging (Haaker et al., 2019). Yet, it is important to bridge that gap, as methods in animal research can be more invasive and, therefore, be more precise regarding experimental manipulations of neural circuitries than methods applicable to human research.

A majority of the insights into the neural fear circuits in humans result from functional neuroimaging studies. By using fear conditioning paradigms, early functional magnetic resonance imaging (fMRI) and positron emission tomography (PET) found increased amygdala activity to the CS+ compared to the CS- (Buchel & Dolan, 2000; Mechias, Etkin, & Kalisch, 2010), demonstrating first evidence that results from animal studies translate to the human brain. Additional activities were identified in the anterior cingulate cortex and motor-related brain regions (Buchel & Dolan, 2000). Initial meta-analyses substantiated the notion of a human 'fear network' with the amygdala as a central node, but also demonstrated involvements of the anterior cingulate and insular cortices, which were interpreted as parts of an autonomic-interoceptive network (Etkin & Wager, 2007; Mechias et al., 2010). Interestingly, the most recent meta-analysis of human fear conditioning studies could not find robust activity in the amygdala, attributing this absence of activation mainly on technical constraints of fMRI (Fullana et al., 2016). However, the authors identified an extended fear network, which comprises brain areas that were already mentioned in earlier imaging

studies, like the anterior insula, anterior cingulate cortex and motor-related areas, as well as regions known from animal studies, e.g. the prefrontal cortex, midbrain areas and thalamic nuclei, indicating the importance of conscious, interoceptive and motivational processes (Fullana et al., 2016).

During extinction learning, neural activity of the fear network gradually declines (Fullana et al., 2018). In addition, fear extinction prompts increased activation of the ventromedial prefrontal cortex (vmPFC) and dorsolateral prefrontal cortex (Milad et al., 2007; Tovote et al., 2015), which are proposed to regulate fear expression (Milad & Quirk, 2012) and suggest the existence of a specific extinction network that actively inhibits the neural circuits underlying fear. Further, these findings indicate distinct fear and extinction memory traces, emphasizing that fear extinction is not just a reduction of conditioned responses but also involves new learning (Milad, Orr, Pitman, & Rauch, 2005). It is important to mention, however, that a recent meta-analysis could not find consistent evidence for vmPFC activity during extinction learning in human fear conditioning (Fullana et al., 2018).

In summary, brain areas identified in human neuroimaging studies only partly overlap with neural fear circuits found in animal studies (Sevenster, Visser, & D'Hooge, 2018). While fMRI studies highlight the role of prefrontal, cingulate and insula cortices, the role of the amygdala in human fear conditioning remains elusive, with some studies finding amygdala activity and some not.

These inconsistencies might be due to methodological reasons. For example, the amygdala consists of many different subnuclei, of which some could show hyperactivation and some could show hypoactivation, which might offset because of the insufficient temporal and spatial resolution in human fMRI (Fullana et al., 2016; Morriss, Hoare, & Reekum, 2018). However, the discrepancies could also be explained with the dual-system approach (LeDoux & Pine, 2016), which considers the amygdala as the key hub for autonomic defensive responses but not as the generator of conscious fearful

feelings, which are mediated by more cognitive, fronto-temporal circuits. In line with the dual-system approach, it is important to mention that, for ethical considerations, the aversiveness of the unconditioned stimuli in human research is often lower than in animal research (Haaker et al., 2019). Compared to animals, human participants are usually informed about the US prior to the experiment; during the individual threshold detection procedure they have some control over the US-intensity; and because of that, they are already pre-exposed to the US. These reasons could contribute to the fact that fear conditioning in humans might trigger a different learning pattern than in animals, which relies more on propositional than on associative learning and therefore elicits less amygdala activity. However, it is too early to draw final conclusions, especially with recent advances in animal research and an increased focus on a translational perspective to bridge that gap.

1.3 The role of threat ambiguity on the organization of defense mechanisms

Over the last few decades, research on threat processing has primarily focused on models and situations where the threatening stimulus is easily detectable and clearly dangerous. As seen above, most studies investigating fear conditioning rely on threat stimuli that are distinctly related to the conditioned stimulus. However, recent studies demonstrate that the ambiguity of the threatening stimulus plays a major role in the organization of threat processing mechanisms (Blanchard, Hynd, Minke, Minemoto, & Blanchard, 2001). Accordingly, threat can either be very specific (actual threat) or highly diffuse and uncertain (potential threat) (Davis, 1992).

Following the terminology of the neuroscience literature, actual threat elicits a phasic response, which is present-oriented and quickly dissipates once the threat has past; while potential threat is associated with a more future-oriented, sustained re-

sponse (Davis, Walker, Miles, & Grillon, 2010; Sylvers, Lilienfeld, & LaPrairie, 2011). On a behavioral level, animal studies demonstrated that actual threat typically triggers fight, flight, or freezing responses, whereas potential threat prompts risk assessment and approach behavior (Blanchard et al., 2001; Sylvers et al., 2011). Moreover, acute and potential threat can also be separated on a continuum of temporal and spatial distance to a predator (Fanselow, 1994), forming the basis of the threat-imminence model (Fanselow & Lester, 1988).

1.3.1 Threat-imminence model

The threat-imminence model divides the organization of defensive behavior into three stages (see Fig. 1.1A), corresponding to the organism's temporal or spatial distance to a threat (Blanchard & Blanchard, 1989; Fanselow & Lester, 1988). Crucially, as human defensive behaviors parallel patterns of animal behavior, the threat-imminence model has already been adapted to investigate threat processing mechanisms in humans (Lang, Bradley, & Cuthbert, 1997; Lang, Davis, & Ohman, 2000). During the *pre-encounter* stage, the organism has entered an area, where it, for example, notices the odor of a predator or has already encountered threat in the past. Actual threat, however, has not yet been identified, resulting in a situation of potential threat. Typical pre-encounter behavior in rodents include careful approach activities, which can be interpreted as risk-assessment, and heightened vigilance with the goal to resolve uncertainties in the environment. In humans, this is paralleled by enhanced orientation responses (Bradley, Keil, & Lang, 2012), while a general activation is reflected in mild levels of physiological arousal (Lang et al., 2000). Because of the potentially dangerous and uncertain character, the pre-encounter stage has often been related to a feeling of anxiety (Fanselow, 2018).

Once a threat in the present environment has been identified, the organism enters the *post-encounter* stage. During this stage, freezing is the most dominant behavior in

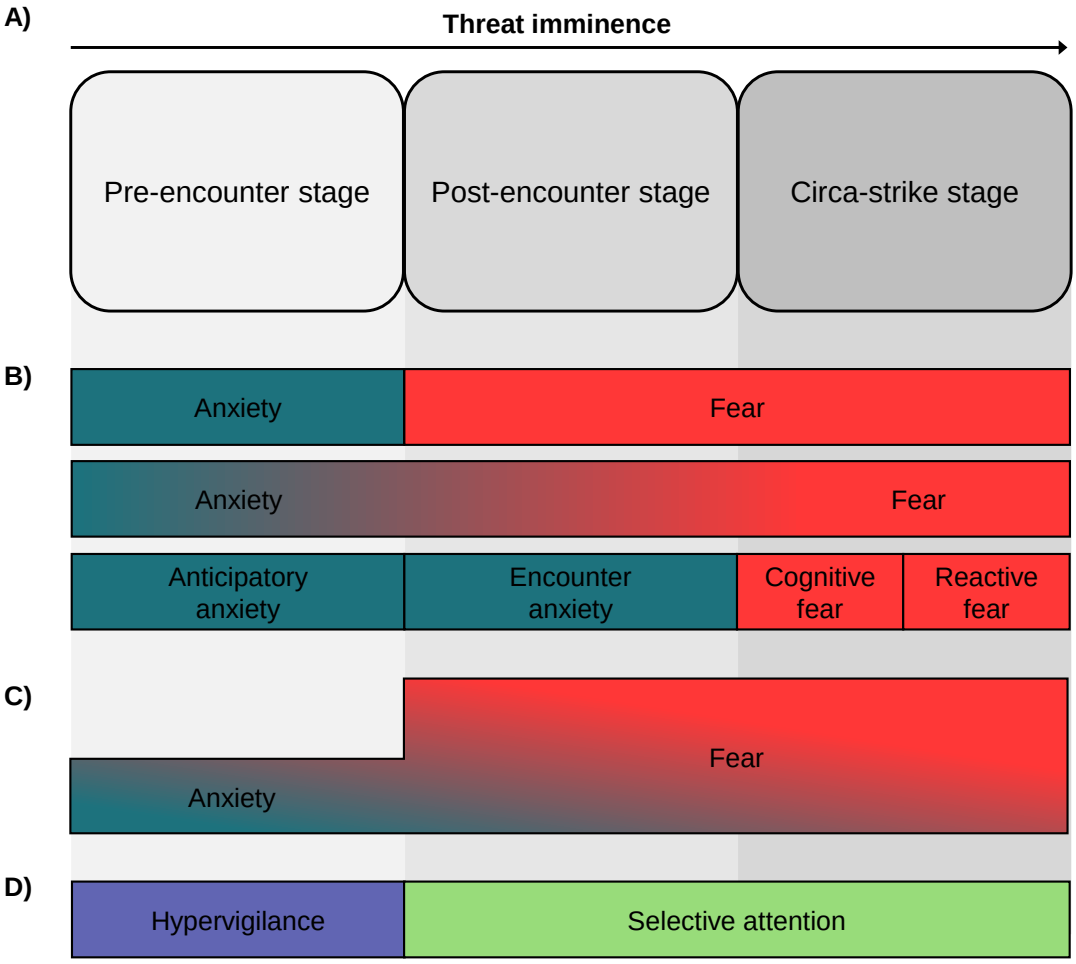

Figure 1.1: A) The three stages of the threat-imminence model. B) Different conceptions of how fear and anxiety map onto the threat-imminence-continuum. The upper row illustrates a 'strict-segregation' model, where anxiety is associated with the pre-encounter stage, while the post-encounter triggers a switch to fear (e.g. Fanselow, 2018). The middle row depicts a similar idea as a continuum between anxiety and fear instead of discrete emotions. The lower row shows a more fine-grained classification of the emotional states in relation to the threat-imminence model (Mobbs, 2018). C) An illustration of a potential interaction between fear and anxiety. In this example, anxiety potentiates concurrent fear responses. D) Functions of attentional processing in relation to different stages of the threat-imminence model (Lang et al., 2000).

order to reduce the likelihood of detection. However, the organism is also preparing for an upcoming fight-or-flight behavior and physiological arousal progressively increases

(Lang et al., 1997). It has been suggested that human heart rate deceleration (fear bradycardia) and startle-response inhibition to mildly threatening stimuli parallel animal freezing behavior (Lang et al., 2000). As a direct index of freezing-like behavior in humans, Roelofs et al. (2010) have also demonstrated that threatening stimuli induce a reduction of body sway, which was associated with bradycardia. More recent studies have further demonstrated freezing-like patterns of eye movements during the anticipation of aversive events in human subjects (Merscher & Gamer, 2020; Rösler & Gamer, 2019). During the post-encounter stage, the detection of an actual threat prompts fear instead of anxiety (Fanselow, 2018).

The final, *circa-strike* stage comprises the event of an attack or the inevitable contact with the threat where the actual fight-or-flight response becomes necessary. Heart rate acceleration and startle potentiation are characteristic for the circa-strike stage and physiological arousal is further potentiated (Lang et al., 1997). Since survival is at stake, behavior is dominated by hard-wired species-specific defensive reactions (Blanchard & Blanchard, 1989). In humans, the feeling of fear increases (Fanselow, 2018).

Critically, the threat-imminence model is the only model in the neuroscience literature that incorporates the emotional states of both fear and anxiety and relates them on a continuum of temporal and spatial distance to a threat (Fanselow, 1994). According to the APA (2013), fear is defined as the emotional response following real or perceived imminent threat, while anxiety is described by the anticipation of unspecific, future threat. Until now, however, there is no consistent distinction between fear and anxiety (Perusini & Fanselow, 2015). In everyday life, the terms are often used interchangeably, and even clinical psychologists seldom differentiate between them. The lack of distinction is compounded by the fact that fear and anxiety share underlying mechanisms to some extent and also seem very similar regarding observable behavior (Shackman & Fox, 2016).

Therefore, the threat-imminence model led to tremendous progress in the field of fear and anxiety research. In a more modern adaption of the threat-imminence model, Mobbs et al. (2015) integrated novel cognitive and learning theories and proposed a human model of threat processing mechanisms in relation to predatory proximity. In short, depending on the imminence of a threat, the human survival optimization system responds with different survival strategies, like predicting, threat orienting and assessment, or defensive strategies, which are further modulated by cognitive (re-)appraisal and control systems (for more detail, see Mobbs et al., 2015). Similarly, the authors incorporated the emotional concepts of fear and anxiety that map onto the different stages of their model (Fig. 1.1B) (Mobbs, 2018; Mobbs, Headley, Ding, & Dayan, 2020). The pre-encounter stage is associated with anticipatory anxiety, while in contrast to the original threat-imminence model, threat encounter does not elicit fear but ('encounter') anxiety. Both forms of anxiety are characterized by 'what-if' cognitions and an apprehensive feeling of uncertainty, however they differ regarding the specificity of their source. The circa-strike stage is further divided into the physical contact with the actual threat and the period before and after the attack, when there is still time to strategize. While the former elicits reactive fear, which encompasses the classic fight-or-flight response, the latter is associated with cognitive fear, which is defined as a 'conscious feeling of terror' (Mobbs, 2018).

As outlined above, different ideas exist how fear and anxiety map onto the stages of the threat-imminence model (Fanselow, 2018; Mobbs, 2018). Even more strikingly, the transition from anxiety to fear has not yet been considered. However, the literature seems to agree on anxiety being more associated with potential threat, while fear is triggered by acute threat. Accordingly, fear and anxiety might be the anchor points of an emotional continuum that closely relates to threat proximity. Yet, qualitative differences and the involvement of distinct neural circuits suggest two separate emotional states, rather than a single continuum between fear and anxiety (Fox &

Shackman, 2019; Sylvers et al., 2011). This seems to be the prevailing view in the neuroscience literature and has even been incorporated into the US National Institute of Mental Health Research Domain Criteria (RDoC; Cuthbert, 2014; Insel, 2014; and Insel et al., 2010). Critically, prevailing models assume that fear and anxiety are mutually exclusive and that only one of both states is active at a time. Yet again, no consensus has been reached on when the transition between fear and anxiety takes place (Fig. 1.1B). Furthermore, recent studies raised criticism regarding their separation and even emphasized a potential interaction between fear and anxiety (Fox & Shackman, 2019; Hur, Stockbridge, Fox, & Shackman, 2019; Shackman & Fox, 2016).

However, due to a lack of suitable paradigms, direct evidence for an interaction is scarce. In addition, the absence of a mechanistic account for the interaction of fear and anxiety stalls neuroscientific progress. To fill this gap, it is important to consider the functional relevance of anxiety for fear and vice versa. Assuming that potential threat elicits anxiety and acute threat prompts fear (Davis et al., 2010), the question arises, if fear responses to threatening stimuli depend on whether they are encountered during situations of potential threat or not. Accordingly, the present thesis tests the hypothesis that anxiety potentiates concurrent fear responses, suggesting that fear and anxiety interact with each other in order to facilitate adaptive behavior (also see Fig. 1.1C).

1.3.2 Contextual anxiety conditioning

To investigate the underlying mechanisms of fear and anxiety, acute and potential threat need to be implemented in the laboratory. As seen above, acute threat can be modeled with fear conditioning paradigms, whereas context conditioning is a good model for potential threat situations.

During context conditioning, aversive stimuli are presented in the absence of any signaling cues (Glotzbach-Schoon, Andreatta, et al., 2013). Consequently, the context

(anxiety context, CTX+) in which the aversive stimuli are presented becomes the best predictor for the US (Baas, Ooijen, Goudriaan, & Kenemans, 2008). However, since individuals are not able to predict when the aversive events occur, a potential threat situation arises, leading to a sustained state of anxious apprehension (Grillon, 2002).

While it is relatively easy to describe discrete stimuli used in fear conditioning paradigms, a definition of contexts is more complex. While spatial (e.g. places) and temporal (e.g. time reference) factors serve as clearly identifiable contexts, interoceptive (e.g. stress) or cognitive contexts (e.g. a state of mind induced via verbal instructions) are more difficult to grasp (Maren, Phan, & Liberzon, 2013). In general, contexts can be broadly defined as the set of circumstances that surround an event (Maren et al., 2013). Accordingly, there are many possibilities to induce contexts that enclose aversive events and successful context conditioning has been demonstrated with various types of context stimuli, e.g. background colors (Lang et al., 2009; Vansteenwegen, Iberico, Vervliet, Marescau, & Hermans, 2008), rooms in virtual reality (Andreatta, Glotzbach-Schoon, et al., 2015; E. Glotzbach et al., 2012), geometrical symbols (Wieser et al., 2016b), or verbal instructions (Grillon, Baas, Lissek, Smith, & Milstein, 2004) that were presented on monitor screens. Notable factors that delineate context from cue conditioning are context duration and timing of the US delivery. Contexts are usually presented for a longer interval than discrete cues, although no consensus on the optimal duration has been reached with studies using context intervals ranging from 20 seconds (Kastner, Pauli, & Wieser, 2015) to eight minutes (Davis et al., 2010). However, in contrast to cue conditioning, where the US is almost always presented shortly after the offset of the cue, the USs occur at random time points during the context presentation in context conditioning to induce uncertainty and potential threat.

Context conditioning is just one method to induce potential threat. Other methods of equal value are, for example, threat-of-shock paradigms (Bublatzky, Gerdes,

& Alpers, 2014), where one context is instructed to be associated with an aversive event although it does not occur throughout the experiment or paradigms that induce uncertainty by varying the temporal delay between a cue and the US (Herrmann et al., 2016).

Exposure to the anxiety context compared to a neutral context is associated with potentiated responses in somato-visceral measures, like skin conductance level (Glotzbach-Schoon, Andreatta, Muhlberger, & Pauli, 2015) and fear-potentiated startle (Andreatta et al., 2015; Glotzbach-Schoon, Andreatta, et al., 2013; Glotzbach-Schoon, Tadda, et al., 2013), as well as increased verbal reports of arousal and anxiety (Glotzbach et al., 2012) and therefore, parallels results of fear conditioning studies. However, the main differences between the readouts of fear and anxiety mainly stem from studies investigating the neural responses during cue and context conditioning.

1.3.3 Neural correlates of anxiety

Recently, scientists in animal and human research alike tried to uncover the neural network underlying anxiety. As described earlier, it has been suggested that the central amygdala (CeA) is the central hub in the neural fear circuit. Using potential threat paradigms, studies in rodents demonstrated that the bed nucleus of the stria terminalis (BNST) plays an equivalent role for the neural anxiety network (Davis et al., 2010; Tovote et al., 2015). The BNST receives projections from the basolateral and central nuclei of the amygdala and resembles the amygdala regarding cell types and output projections (Davis et al., 2010). It is therefore often considered as part of the so-called 'extended amygdala'. Like the central amygdala, the BNST projects to hypothalamic and brainstem regions that control autonomic and behavioral threat responses.

Based on lesion and pharmacological methods in rodents, it was shown that deactivating the CeA dampened responses to acute threat, while deactivating the BNST

was associated with decreased anxiety-like behavior and responses to potential threat, suggesting independence between the neural networks of fear and anxiety (Davis et al., 2010). However, the CeA and BNST do not only share input and output projections but they are also highly interconnected themselves. For example, the basolateral amygdala projects to the BNST as well as the medial and lateral part of the CeA. In addition, there are connections from the lateral part of the CeA to the BNST (Davis et al., 2010), and most importantly, the BNST has re-entrant projections to the medial part of the CeA. These results do not only demonstrate a substantial overlap between the circuits underlying potential and acute threat but also lay out the neural foundation for an interaction between fear and anxiety (Tovote et al., 2015).

First studies on the structural and functional connectivity of the BNST in humans showed converging results across human and animal research (Avery et al., 2014). Furthermore, fMRI-studies found potentiated blood oxygenation level dependent (BOLD-)responses to potential threat in the BNST (Alvarez, Chen, Bodurka, Kaplan, & Grillon, 2011; Somerville, Whalen, & Kelley, 2010). However, recent human fMRI-studies demonstrated that neural activity during potential threat is not limited to BNST responses but also includes increased amygdala and hippocampal activity (Andreatta, Glotzbach-Schoon, et al., 2015; Gorka et al., 2017a; Lonsdorf, Haaker, & Kalisch, 2014). These findings are in contrast with a functional dissociation between the CeA and the BNST for the expression of fear and anxiety (Shackman & Fox, 2016). Indeed, recent studies in humans and nonhuman-primates found that the CeA and the BNST are both engaged by potential as well as acute threat (Fox, Oler, Tromp do, Fudge, & Kalin, 2015; Fox & Shackman, 2019; Shackman & Fox, 2016). These results substantiate the idea of an interaction model of fear and anxiety and re-raise the questions, which were initially discussed in the context of the threat-imminence-model: What are the aspects that delineate fear from anxiety and if they are not independent, which mechanisms characterize their interaction? A promising

approach to address these questions is by leveraging the well-established differences in attentional processing between fear and anxiety.

1.4 Visual perception and attention to threat

1.4.1 Attentional mechanisms during acute and potential threat

It has long been noted that attentional mechanisms play a major role in the processing of threat and are therefore frequently discussed in the context of fear and anxiety (Bar-Haim, Lamy, Pergamin, Bakermans-Kranenburg, & van, 2007; Bradley et al., 2012; Mogg & Bradley, 1998; Öhman, Flykt, & Esteves, 2001; Sokolov, 1963). Specifically, anxiety is often characterized as a prolonged state of heightened vigilance (Davis et al., 2010; Sylvers et al., 2011). This is in line with conceptual models that focus on the functional differences of attention. For example, Richards et al. (2014) proposed a distinction between hypervigilance and selective attention in relation to potential and acute threat. In this framework, hypervigilance is defined as a broadening of attention or an excessive and rapid scanning of the environment with the goal to enhance threat detection in situations of potential threat. In contrast, selective attention characterizes a narrowing of the attentional focus on the threatening stimulus once it has been identified. The purpose of selective attention is to closely monitor the source of the threat and to ensure that it receives processing priority over non-threatening stimuli (Mogg & Bradley, 1998).

Accordingly, Lang et al. (2000) related the stages of the threat-imminence model to the different functions of attentional processing (see also Fig. 1.1D). In the threat-imminence model, the crucial event comprises the transition from pre- to post-encounter stage, after the threatening stimulus has been detected. A rapid detection

of threat increases the organism's chance for survival (Öhman et al., 2001). Consequently, the main mode of attention during the pre-encounter stage is hypervigilance. During the post-encounter stage, attentional processing of the threatening stimulus is prioritized and ongoing activities are interrupted. Thus, threat-encounter prompts selective attention to enhance defensive responding and to prepare for an upcoming circa-strike event.

The overarching aim of both hypervigilance and selective attention is to facilitate adaptive behavior. For that reason, a disruption of these abilities has been discussed as a major factor in the development and maintenance of pathological fear and anxiety.

1.4.2 Attentional biases in anxiety disorders

A large number of studies suggested the involvement of attentional biases in anxiety disorders (Bar-Haim et al., 2007; MacLeod & Rutherford, 1992; Mathews, May, Mogg, & Eysenck, 1990; Mogg & Bradley, 1998), of which a majority demonstrated that anxious individuals are characterized by heightened attention towards threat-related stimuli (Bar-Haim et al., 2007). Importantly, threat-related attentional biases can be found in virtually all types of anxiety disorders (Cisler & Koster, 2010). In fact, there are many different theories attempting to explain the mechanisms underlying these biases. While some of them highlight the evolutionary significance and focus on the neural circuits (Öhman, 2005; Öhman & Soares, 1993), others emphasize motivational (Bar-Haim et al., 2007; Mogg & Bradley, 1998) and cognitive mechanisms (Eysenck, Derakshan, Santos, & Calvo, 2007; Mathews & Mackintosh, 1998; Wells & Matthews, 1994). Accordingly, there is a lack of agreement regarding the underlying mechanisms and neural components, as well as the stage of attentional processing in which the dysfunction occurs. Some studies even found opposing results, with diminished attention toward threat-related stimuli in anxious individuals (Chen, Ehlers, Clark, & Mansell, 2002; Ruiter & Brosschot, 1994). In addition, a

recent meta-analysis of behavioral studies was not able to find evidence for threat-related attentional biases in clinical anxiety at all (Kruijt, Parsons, & Fox, 2019).

This lack of converging evidence may be related to the fact that the majority of these results stem from studies that quantify attention allocation by the means of reaction-time tasks (Bar-Haim et al., 2007; Kruijt et al., 2019). In particular, various different versions of Stroop tasks (Ruiter & Brosschot, 1994; Stroop, 1935), visual-search tasks (Brosch & Sharma, 2005; Öhman et al., 2001), spatial cuing tasks (Fox, Russo, Bowles, & Dutton, 2001; Posner, 1980), or dot-probe paradigms (Bradley, Mogg, Falla, & Hamilton, 1998; MacLeod, Mathews, & Tata, 1986) have been used to investigate attentional biases in anxious individuals. Even though these paradigms seem to be well established in psychological research, a growing body of literature raises concerns regarding the reliability and validity of these tasks (Bantin, Stevens, Gerlach, & Hermann, 2016; Dresler et al., 2012; Kruijt et al., 2019; Schmukle, 2005; Strauss, Allen, Jorgensen, & Cramer, 2005; Thigpen et al., 2018b). Ultimately, the use of these indirect measures of attention are not able to prove the existence of threat-related attention biases in anxiety disorders. Therefore, to unravel attentional and perceptional mechanisms in anxiety, it is necessary to provide direct evidence from a neurophysiological level for threat-related attention (for a review, see Wieser & Keil, 2020).

1.4.3 Neural correlates of visual perception

The neural structure of the visual system is one of the most investigated area in the neuroscience literature (Carrasco, 2011). Visual processing starts with the eye, where photoreceptive cells of the retina receive visual input in the form of light. A majority of the retinal output streams to the lateral geniculate nucleus of the thalamus and from there to the primary visual cortex (also called striate cortex or V1). Neurons in the primary visual cortex follow a retinotopic organization (Horton & Hoyt, 1991) and

are highly selective for orientation, spatial frequency, velocity or color (e.g. Carandini et al., 2005; Van Essen, Anderson, & Felleman, 1992). Accordingly, the main function of the primary visual cortex is to encode low-level features of the visual stimulus and to filter input information for further processing (Zhaoping, 2019).

Output signals of V1's neurons are forwarded to many higher-order visual cortical areas (Van Essen et al., 1992). The major output is separated into a ventral and a dorsal stream, which project to the inferotemporal cortex and parietal regions, respectively (Goodale & Milner, 1992; Mishkin, Ungerleider, & Macko, 1983). Several models have been posited to relate these streams to different functions of the visual system, e.g. object identification versus localization (Mishkin et al., 1983) or perception versus action (Goodale & Milner, 1992). However, the idea that these streams are completely functionally independent was refuted by the fact that there is extensive crosstalk between ventral and dorsal pathways (e.g. Schenk & McIntosh, 2010). Ultimately, the visual system distributes sensory information to a widespread network of brain regions, including subcortical structures like the amygdala (Tamietto, Pullens, Gelder, Weiskrantz, & Goebel, 2012).

Recent studies could demonstrate that threat enhances the C1 component, one of the earliest visual ERPs in the primary visual cortex, suggesting that sensory threat processing arises as early as 50 - 70 ms post stimulus onset (Miskovic & Keil, 2012). These findings were in line with traditional theories, stating that threat is processed by a specialized, encapsulated neural circuitry. In these models, sensory information from thalamic or midbrain nuclei are directly forwarded via the so-called 'low-road' to the neural fear network centered around the amygdala and limbic areas like the hippocampus and hypothalamus, where the emotional output is generated (Davis, 1992; LeDoux, 2000). This pathway is supposed to bypass the slower routes of the cortex to increase processing speed of threatening stimuli (LeDoux, 1996). At the same time, the primary visual cortex receives modulatory feedback signals from

higher visual and other brain regions (Lamme, Supèr, & Spekreijse, 1998). These re-entrant signals could provide the framework for a neural implementation of attentional processes. Indeed, there are extensive projections from the amygdala into the visual cortices (Amaral, Behniea, & Kelly, 2003), indicating that the amygdala has substantial modulatory influence over visual processing and - given its key role in fear learning - suggesting a potential mechanism that could drive threat-related attention (Amaral et al., 2003; Miskovic & Keil, 2012; Sabatinelli, Lang, Bradley, Costa, & Keil, 2009). However, novel findings indicated that subcortical visual processing is not significantly faster than cortical processing (Pessoa & Adolphs, 2010). Therefore, recent theories suggest that early sensory processing of threat recruits multiple parallel pathways beyond the subcortical-amygdala pathway, which operate in waves of activation across the visual processing hierarchy (Pessoa & Adolphs, 2010). In addition, it has been noted that feedback projections from the amygdala to sensory cortices is not the only mechanism underlying threat-related attention. The sensory cortical model of threat perception holds that acquired threat representations are already stored in the sensory cortex. Subsequent, encounters with these threats will then activate these representations and initiate threat encoding (Li, 2014).

EEG- and fMRI-studies investigating threat-related attention during fear conditioning showed converging evidence for enhanced visuocortical responses to features related to the CS+ in comparison to the CS- (Boylan, Kelly, Thigpen, & Keil, 2019; Keil, Stolarova, Moratti, & Ray, 2007; Pizzagalli, Greischar, & Davidson, 2003; Shalev, Paz, & Avidan, 2018; Stolarova, Keil, & Moratti, 2006; Wieser et al., 2014a). This threat-related sensory facilitation is accompanied by a cascade of changes in the visual cortex (for a review see Miskovic & Keil, 2012): As stated above, brain regions involved in the fear network, like the amygdala, insular cortex and prefrontal regions, exert modulatory influence via re-entrant connections, leading to a rapid enhancement in sensory processing (Gilbert & Sigman, 2007; Keil et al., 2007). In addition, during

extended phases of aversive learning, adaptations in the local sensitivity of neurons in the primary visual cortex take place in order to facilitate low-level processing of threat-related features (Miskovic & Keil, 2012). These adaptations might be driven by plastic changes of the synaptic connectivity within the visual cortex (Gilbert, Sigman, & Crist, 2001). In line with the cortical model of threat perception (Li, 2014) and given the functional architecture of the primary visual cortex and its highly selective neurons, it also seems possible that neurons in the visual cortex sharpen their tuning functions towards specific threat-related features (McTeague, Gruss, & Keil, 2015). Evidence for this threat-related 'tuning' has already been found for a population of orientation-selective neurons in the V1 (Antov, Plog, Bierwirth, Keil, & Stockhorst, 2020; McTeague et al., 2015). It is up to future work to disentangle these mechanisms and characterize their interplay among the cascade of threat-related changes in the visual cortex.

In conclusion, there is tremendous progress in the study of threat-related sensory changes during fear conditioning. However, the neurophysiological substrate of attention during context conditioning (and potential threat more generally) is still obscure. There are two main reasons for this lack of evidence. First, the event-related potential (ERP) technique that is commonly applied in EEG studies relies on many trials in order to reduce random noise (Huffmeijer, Bakermans-Kranenburg, Alink, & Ijzendoorn, 2014). Often more than 30 trials per condition are required to obtain reliable signal-to-noise ratios (Thigpen, Kappenman, & Keil, 2017). This leads to an inherent problem in context conditioning paradigms, where a single trial usually lasts longer than 30 seconds. Thus, context conditioning ERP studies would be very time-consuming and difficult for the participant to endure, especially regarding attentional processes. Second, time-averaged ERPs quantify only the transient response to a stimulus at its onset. The focus in context conditioning however lies on sustained responses during the context presentation. In sum, the ERP technique is little suited

to investigate sensory processing during potential threat induced by context conditioning. To overcome this issue, recent studies used an ongoing rhythmic stimulation of the visual system to evoke steady-state visual potentials.

1.4.4 Steady-state visual evoked potentials

Steady-state visual evoked potentials (ssVEPs) are oscillatory, electrocortical responses to stimuli that are periodically modulated in terms of luminance (e.g. flickering) or contrast (Norcia, Appelbaum, Ales, Cottereau, & Rossion, 2015; Vialatte, Maurice, Dauwels, & Cichocki, 2010). Importantly, the frequency of the neural oscillatory response equals that of the driving stimuli (Regan, 1989). Critically, since the driving frequency is well known, the ssVEP signal can be reliably separated from random noise, resulting in a robust signal-to-noise ratio (Wang, Clementz, & Keil, 2007), even on the level of single trial analysis (Keil et al., 2008; Thigpen et al., 2018a; Wieser et al., 2014b). A successful induction of ssVEP signals has already been demonstrated over a wide range of driving frequencies (Gruss, Wieser, Schweinberger, & Keil, n.d.; Norcia et al., 2015) and with different types of stimuli, including sinusoidal gratings (so called Gabor-patches; e.g. Miskovic & Keil, 2013b; Keil et al., 2007; Moratti, Keil, & Miller, 2006; Wieser & Keil, 2011), geometrical symbols (Miskovic & Keil, 2014; Wieser et al., 2016b), random dot kinematograms (Boylan et al., 2019; Muller et al., 2006), facial stimuli (Ahrens, Muhlberger, Pauli, & Wieser, 2015; McTeague, Shumen, Wieser, Lang, & Keil, 2011; Wieser et al., 2011, 2014b) and pictures of affective scenes (Bekhtereva, Pritschmann, Keil, & Müller, 2018; Keil et al., 2003; Keil, Moratti, Sabatinelli, Bradley, & Lang, 2005; Wieser & Keil, 2014).

The ssVEP signal is assumed to originate from the primary visual cortex and reflects neural activity on the populational level (Di Russo et al., 2007). Thus, the amplitude of the driving frequency represents the degree of visuocortical activation and can be quantified by transforming the scalp-recorded ssVEP signal into the frequency-

domain. Various studies demonstrated that the amplitude of the ssVEP is sensitive to attentional tasks and features. For instance, attended features (Muller et al., 2006) and locations (Muller, Teder-Salejarvi, & Hillyard, 1998) enhance visuocortical activation compared to unattended features and locations. In addition, ssVEPs are sensitive to affective features and show increased amplitudes for emotional compared to neutral stimuli (Keil et al., 2003, 2005; McTeague et al., 2011; Wieser & Keil, 2014).

In this growing literature, the ssVEP technique has already been used to study sensory processing during fear conditioning. These studies found unanimous evidence for enhanced ssVEP amplitudes to the CS+ compared to the CS-, indicating facilitated visuocortical engagement to threat-signaling stimuli (Miskovic & Keil, 2013b, 2013a, and see 2012 for a review; Wieser et al., 2014a, 2014b). Leveraging the benefits of the high signal-to-noise ratios, single trial analysis showed increased ssVEP amplitudes to the CS+ already after as few as two pairings with the aversive event (McTeague et al., 2015). In addition, it could be shown that changes in visuocortical processing during fear conditioning is primarily driven by experience, but not by conscious expectancy (Moratti & Keil, 2009; Moratti et al., 2006). In fact, a recent study provided evidence that ssVEP amplitudes during aversive learning follow a function of associative strength as predicted by a computational (Rescorla-Wagner) model (Yuan et al., 2018).

In addition to the robust signal-to-noise ratio, there are two major advantages of the ssVEP method over transient ERP methods. First, the neural responses are entrained as long as the driving stimulus is presented, making ssVEPs an optimal tool to study sustained sensory processing during context conditioning. Second, ssVEPs separately quantify visuocortical engagement to two or more simultaneously presented and spatially overlapping stimuli, if they are presented at different frequencies (frequency tagging; e.g. Wieser et al., 2011). This technique not only allows a quantifi-

cation of attention allocation to similar stimuli competing for attentional resources (Wieser et al., 2011), but also enables a disentangling of the visuocortical responses to a foreground and a background cue (Wieser & Keil, 2014). Thus, ssVEPs and the frequency tagging method offer a unique opportunity to compare visuocortical activity to visual cues in fear and context conditioning paradigms.

1.5 Summary and research questions

The overarching goal of this thesis is to elucidate attentional processes during fear and anxiety. Following the assumptions of the threat-imminence model, fear is induced by acute threat, while anxiety arises during potential threat. Yet, the exact boundaries between fear and anxiety are not well defined. In fact, conceptual frameworks and theories focusing on the neural networks even raise the hypothesis of a potential interaction between fear and anxiety.

Crucially, fear and anxiety are supposed to be characterized by specific attentional mechanisms. Acute threat prompts selective attention, while potential threat is associated with hypervigilance. However, a neurophysiological account of selective attention and hypervigilance is lacking. To fill this gap, this thesis exploits the benefits of steady-state visual evoked potentials as a direct measure of visuocortical activation.

In sum, four experiments were run to address three fundamental questions regarding the interaction of fear and anxiety and the function of attention therein.

First, Study 1 investigates steady-state visual evoked potentials during a combined cue and context conditioning paradigm in order to establish a visuocortical correlate of selective attention and hypervigilance during acute and potential threat.

Second, Study 2 and 3 utilize ssVEPs in addition to psychophysiological measures of arousal to test two alternative hypotheses regarding the interaction of fear

and anxiety: A potential interaction would be reflected through potentiated fear responses during anxiety compared to neutral contexts. By contrast, if anxiety does not influence cortical or psychophysiological responses to fear cues, there will be no interaction.

Third, Study 4 tests the boundary conditions of steady-state visual evoked potentials as a measure of sustained attention during potential threat by using flickering video stimuli of virtual environments. This study is intended to bridge the gap to other context conditioning studies using more complex stimuli that represent the dynamic visual environment outside of the laboratory.

Study 1: The effect of trait anxiety on attentional mechanisms in combined context and cue conditioning and extinction learning

This study has been partly published in *Scientific Reports*. (2019). Stegmann, Y., Reicherts, P., Pauli, P. and Wieser, M. J..

2.1 Introduction

The threat-imminence model suggests different roles of attentional processing as a function of the temporal and spatial distance to a predator (Blanchard & Blanchard, 1989; Fanselow & Lester, 1988; Lang et al., 2000). Accordingly, potential threat is closely linked to heightened vigilance in order to enhance threat detection, while acute threat prompts selective attention, resulting in a prioritized processing of the threatening stimulus (Richards et al., 2014). However, due to a lack of suitable methods and paradigms, a direct comparison of the neural correlates of selective attention and hypervigilance is still missing.

Recently, studies have used the NPU-threat task, which combines cue and context conditioning to compare acute versus potential threat in the laboratory (Grillon et al., 2004; Schmitz & Grillon, 2012). The NPU-threat task typically consists of three different context conditions: During the predictable condition (P) aversive events are

administered at the offset of a signaling cue, inducing acute threat. During the unpredictable condition (U), aversive events occur randomly, modelling aspects of potential threat. In the neutral condition (N), there are no aversive events. Each condition is indicated by verbal instructions about the upcoming contingency and contains up to three brief presentations of a centrally presented visual cue. Importantly, this central cue reliably signals aversive events in the P-condition only. The original version of the NPU-threat task includes three N, two P and two U conditions, with each presentation usually lasting about 120 seconds (Schmitz & Grillon, 2012), but also versions with alternative trial structure and timing exist (e.g. Haaker, Lonsdorf, Thanellou, & Kalisch, 2013; Alvarez et al., 2011; Nelson et al., 2015a).

The NPU-threat task has already been established using a wide range of dependent measures, including ratings of fear and anxiety (Grillon, Baas, Cornwell, & Johnson, 2006; Grillon et al., 2004; Haaker et al., 2013), skin conductance responses (Grillon et al., 2004; Haaker et al., 2013), cardiovascular activity (Grillon et al., 2004; Kastner-Dorn, Andreatta, Pauli, & Wieser, 2018), startle reflexes (Grillon et al., 2006, 2004; Haaker et al., 2013), event-related potentials (Nelson et al., 2015a, 2015b) and fMRI (Alvarez et al., 2011; Kuhn et al., 2016; Lonsdorf et al., 2014). To quantify fear and anxiety, these studies typically compare responses to central cues and responses during contexts among the conditions (e.g. Grillon et al., 2004). In summary, they found converging evidence for fear-potentiated responses to cue presentations in the P-condition and anxiety-potentiated responses during the U-condition.

In a recent study, Wieser et al. (2016b) investigated ssVEPs during an adapted version of the NPU-threat task. The authors exploited the benefits of frequency tagging to separately quantify visuocortical engagement to the central and context cues. In this version of the NPU-threat task, the three conditions were represented by visual context cues, consisting of different geometrical symbols (squares, triangles or circles), which were presented peripherally at each corner of the monitor. Aversive events (US)

were administered in the form of mildly painful electro-tactile stimulation according to the NPU-scheme: US were presented at the offset of the central cue presentation in the P-condition and independent of the central cue in the U-condition. No US were presented during the N-condition. Crucially, the context and central cues were presented in different flicker frequencies to enable a disentangling of the visuocortical responses in the electrocortical signal. Paralleling results of the NPU literature, enhanced ssVEP amplitudes to the central cue were found in the P-condition, while ssVEP amplitudes to the contexts were elevated during the U-condition (Wieser et al., 2016b). Thus, the authors demonstrated first evidence for the electrocortical correlates of selective attention to predictable, acute threat and hypervigilance during unpredictable, potential threat.

Several studies investigating clinical forms of anxiety with the NPU-threat task found enhanced reactivity to potential, but not acute threat for patients with panic disorder (Grillon et al., 2008), PTSD (Grillon et al., 2009), social anxiety disorder and specific phobias (Gorka et al., 2017b). Consequently, aberant responding to potential threat has been discussed as a potential biomarker for pathological forms of fear and anxiety. Interestingly, Carleton (2016) even proposed 'fear of the unknown' (i.e. potential threat) as the fundamental fear underlying all anxiety disorders. Reviewing the neurobiological and psychological literature, Grupe & Nitschke (2013) proposed five psychological key processes that are closely linked to potential threat processing and may contribute to pathological anxiety. In this framework, anxiety is characterized by heightened expectations of danger, which prompt enhanced physiological reactivity in situations of potential threat. These processes result from deficient safety learning and are maintained by behavioral or cognitive avoidance, as well as increased attention and hypervigilance to threat, thus highlighting the important role of attentional processes in anxiety.

To further elucidate the role of attentional mechanisms during potential threat

in the development of anxiety disorders, it is important to examine individuals at risk from these disorders. Since elevated trait-anxiety is an important predisposition to develop pathological anxiety (Barlow, 2004; Chambers, Power, & Durham, 2004), this goal can be achieved by comparing high trait-anxious, healthy participants with average or low trait-anxious participants.

Regarding aversive conditioning, findings on the influence of trait-anxiety are mixed, however, and different results have been reported. For example, trait-anxiety measured by the Spielberger State-Trait Anxiety Inventory (STAI; Spielberger, Gorsuch, & Lushene, 1970) was not associated with discriminatory fear learning in a differential conditioning paradigm (Arnaudova et al., 2013). On the other hand, trait-anxiety was suggested to enhance the general responsiveness to aversive conditioned stimuli (Stegmann, Schiele, et al., 2019). Crucially, recent studies reported that trait-anxiety is positively correlated with responding to potential threat (Baas et al., 2008; Glotzbach-Schoon, Tadda, et al., 2013; Haddad, Pritchett, Lissek, & Lau, 2012). In these studies, potential threat was either induced by unpredictable aversive events during context conditioning (Glotzbach-Schoon, Tadda, et al., 2013), by introducing a novel stimulus that closely resembles the CS+ (Haddad et al., 2012), or by obscuring the predictive value of the CS (Baas et al., 2008). These results substantiate the hypothesis of heightened reactivity to unpredictable threat in anxious individuals. However, no study has yet examined the influence of trait anxiety on visuocortical engagement to acute and potential threat during acquisition and extinction learning.

Therefore, the aim of the present study is threefold: The first goal is to replicate the results of Wieser et al. (2016b) to further substantiate the electrocortical correlates of selective attention and hypervigilance during acute and potential threat. The second goal is to compare high and low anxious individuals regarding these attentional mechanisms. According to previous findings, I expect aberrant visuocortical processing during potential, but not acute threat in high vs. low anxious individu-

als. The final goal is to investigate the stability of this threat-related visuocortical processing during extinction learning. In this regard, I predict reduced extinction learning in high vs. low anxious individuals (Duits et al., 2015).

2.2 Methods and material

2.2.1 Participants

In this study, sixty subjects were recruited via online advertisements and on a local participant-platform. Participants were required to be between 18 and 35 years old, free of any mental or neurological disorders and have no family history of photic epilepsy. Potential participants completed a pre-screening including four items of the STAI-Trait questionnaire that explained the most variance of the STAI-Trait sum-score (Laux & Spielberger, 1981) in a large screening sample (n = 526), which was recruited within the context of the Collaborative Research Centre (SFB-TRR58) at the University of Würzburg (for further details see Schiele et al., 2016). On this basis, a regression analysis was used to estimate the STAI-Trait score to select extreme groups of 30 high and low trait-anxious participants. Cut-off scores were based on the distribution of the local sample, which was highly similar to the original norm samples (Laux & Spielberger, 1981). Accordingly, the cut-offs were ≤ 33 ($\leq 40\%$-quantile) for low trait-anxious and ≥ 41 ($\geq 80\%$-quantile) for high trait-anxious participants. During the experimental session, all participants completed the full STAI-Trait questionnaire (see Table 2.1). The classification could be mainly confirmed, only one subject of each group would have had switched groups and was consequently excluded. Consequently, the final sample consists of 29 high trait-anxious (15 females) and 29 low trait-anxious (15 females) participants. To obtain converging evidence from other questionnaires measuring symptoms of anxiety, participants were required to complete the Anxiety Sensitivity Index 3 (ASI-3; Reiss, Peterson, Gursky, & McNally,

1986), Intolerance of Uncertainty Scale (IUS; Buhr & Dugas, 2002) and the GAD-7 (Spitzer, Kroenke, Williams, & Lowe, 2006). All participants gave written informed consent and were paid 15 € or received an equivalent in course credits. All procedures were approved by the ethics committee of the University of Würzburg.

Table 2.1: Questionnaire data of the low and high anxious groups.

	Low Anxious (n=29)		High Anxious (n=29)		*t*-test	
	M	*SD*	*M*	*SD*	$t(56)$	p
Age	24.62	3.54	25.62	3.41	1.10	.278
STAI-S	33.03	6.04	38.31	5.21	3.56	.001
STAI-T	29.48	4.57	47.41	7.23	11.29	.001
ASI-3	13.45	10.80	22.07	10.96	3.02	.004
IUS	37.62	9.11	53.21	10.18	6.15	.001
GAD-7	2.93	2.15	7.31	3.38	5.88	.001
US-Intensity	2.17	1.37	1.86	1.19	.91	.366
US-Unpleasantness	5.00	.85	5.17	1.39	.57	.571

Note: STAI = State-Trait-Anxiety-Inventory (S: Subscale State, T: Subscale Trait); ASI-3 = Anxiety Sensitivity Index 3; IUS = Intolerance of Uncertainty Scale; US-Intensity in mA.

2.2.2 Materials and Stimuli

Visual stimuli were presented on a 19-inch monitor (resolution = 1024 x 768 pixels) with a vertical refresh rate of 60 Hz, located ca. 100 cm in front of the participant, using the Presentation software (Neurobehavioral Systems, Inc., Albany, CA, USA). Contexts for the different conditions were presented as an array of four triangles,

squares or circles, each spanning a visual angle of 2.4°. The symbols were located in the corners of the monitor ca. 4.7° of visual angle from the center (Fig. 2.1). Central cues were black-and-white Gabor patches, consisting of sinusoidal grating (Gaussian-windowed with maximal contrast at center) with a spatial frequency of 1.4 cycles per degree. The Gabor patches spanned a visual angle of 5.6° horizontally and vertically. Different orientations (-45°, 0° and 45°) were used for the three context conditions. The allocation of visual stimuli to the conditions was counter-balanced across participants.

Unconditioned stimuli (US) were 20 ms electric pulse trains (2 ms pulse width, 25 Hz), which were delivered to the left calf through surface bar electrodes consisting of two gold-plated stainless-steel disks of 9 mm diameter and 30 mm spacing. The US were generated by a constant current stimulator (Digitimer DS7A, Digitmer Ltd., Welwyn Garden City, UK). Prior to the actual experiment, the US were adjusted to the individual pain-threshold (for a protocol, see Andreatta, Muhlberger, Yarali, Gerber, & Pauli, 2010). The mean values of the resulting intensities and pain ratings were 2.2 ± 1.4 mA (mean ± SD) for LA individuals and 1.9 ± 1.2 mA for HA individuals, $t(56)$=0.91, $p = 0.366$, respectively 5.4 ± 0.8 for LA individuals and 5.7 ± 1.5 for HA individuals, $t(56) = 0.86$, $p = 0.394$.

2.2.3 Procedure

After giving written informed consent and completing the questionnaires, participants were seated in a dimly lit, sound-attenuated testing room, where the EEG-net was applied. Then, individual pain thresholds were estimated. The main task was a modified version of the original NPU-threat task (Schmitz & Grillon, 2012), similarly as reported previously (Wieser et al., 2016b). In brief, three different conditions were signaled by the contexts, each lasting for 32 s (one block) and flickering at a frequency of 15 Hz (Fig. 2.1). During every block, two or three central cues were presented for

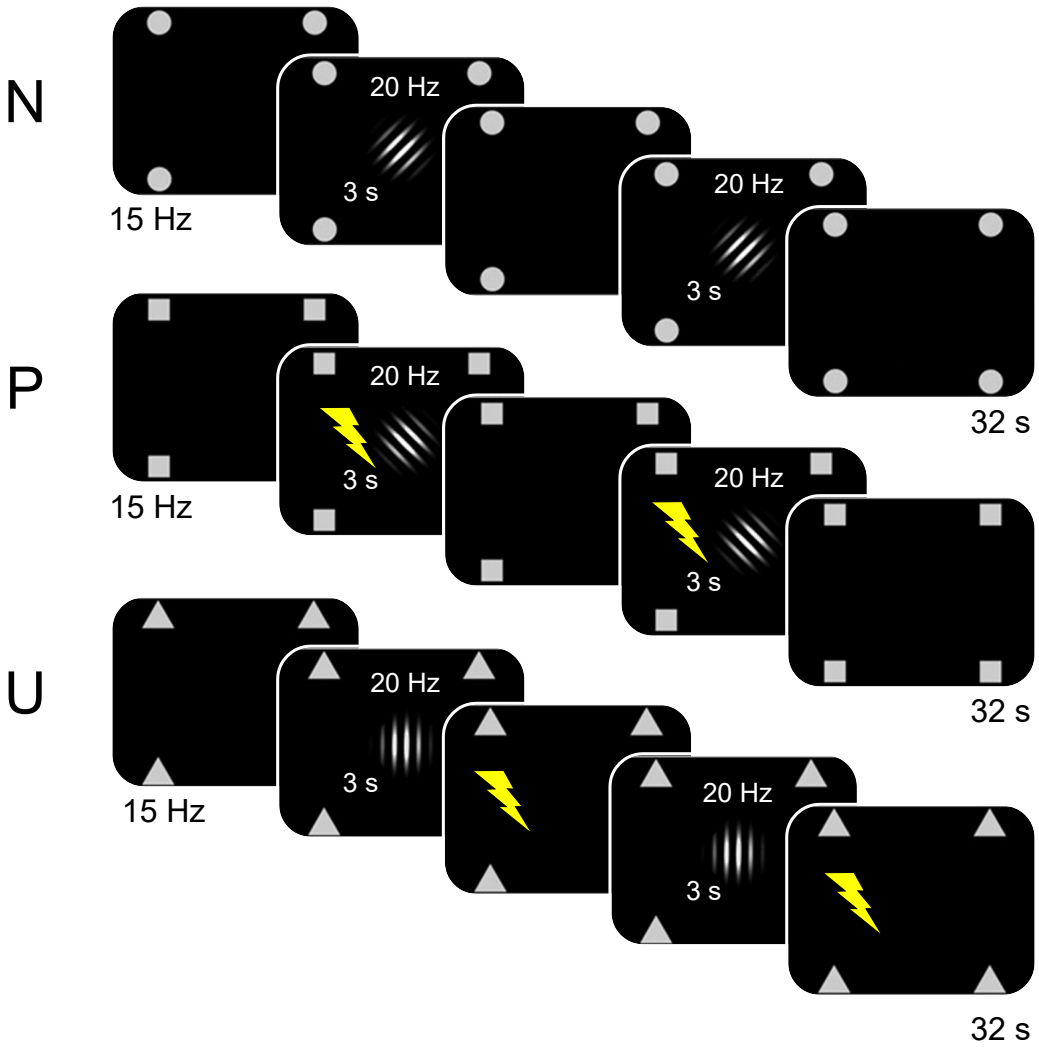

Figure 2.1: Experimental Design. Three context conditions were indicated by different geometrical symbols, presented peripherally for 32 s. At random time points, central cues (-45°, 0°, 45° oriented grating stimuli) were presented for 3 s. To disentangle visuocortical responses evoked by the contexts and central cues, contexts were presented with a flickering frequency of 15 Hz, while central cues were presented in 20 Hz. During acquisition, the central cue reliably predicted US-delivery in the P-condition only, while in the U-condition, US were delivered independently of the central cue. No US were delivered during the N-condition.

3 s, flickering at 20 Hz. Central cues were presented at random time points with an inter-stimulus-interval of at least 3 s. During the first 5 s and last 5 s of each block, no

central cues were presented. In the P-condition, US were presented immediately after the central cue offset (100% reinforcement rate). In the U-condition, two or three US were presented at random time points, but only between 5 s and 30 s after context onset. The interval between two consecutive US was at least 3 s. In addition, US were never administered during a central cue presentation. No US were presented in the N-condition. Paralleling previous NPU-studies, participants were fully instructed about the US-contingency. Since no US were presented during the context onset and central cue presentation in the U-condition, a practice phase was implemented in order to avoid that these periods acquire safety-signal properties. The practice phase consisted of 6 blocks (2 per N, P, U), in which one US in the U-condition was always presented during the central cue presentation and another among the first 5 s after context onset. Then, participants were familiarized with the rating scales. The following acquisition phase consisted of 36 blocks (12 times each condition) with a total of 30 central cue presentations per condition. To obtain the same amount of US during the U- as during the P-condition, a total 30 US were administered during the U-condition. The inter-trial-interval between blocks ranged from 2,500 ms to 3,500 ms. The additional extinction phase, equaled the acquisition phase regarding trial structure and stimulus timing, except US-delivery was omitted. Participants were not instructed about this change of contingencies during extinction.

Ratings of the visual stimuli took place after every 12th block. Participants were asked to rate the contexts and the combination of context and central cue regarding perceived threat ("How threatening do you perceive this picture?", 1 = not threatening at all – 9 = very threatening) and US-expectancy ("To what extent do you expect an electrical stimulus meanwhile this picture is present?", ranging from 0 to 100%).

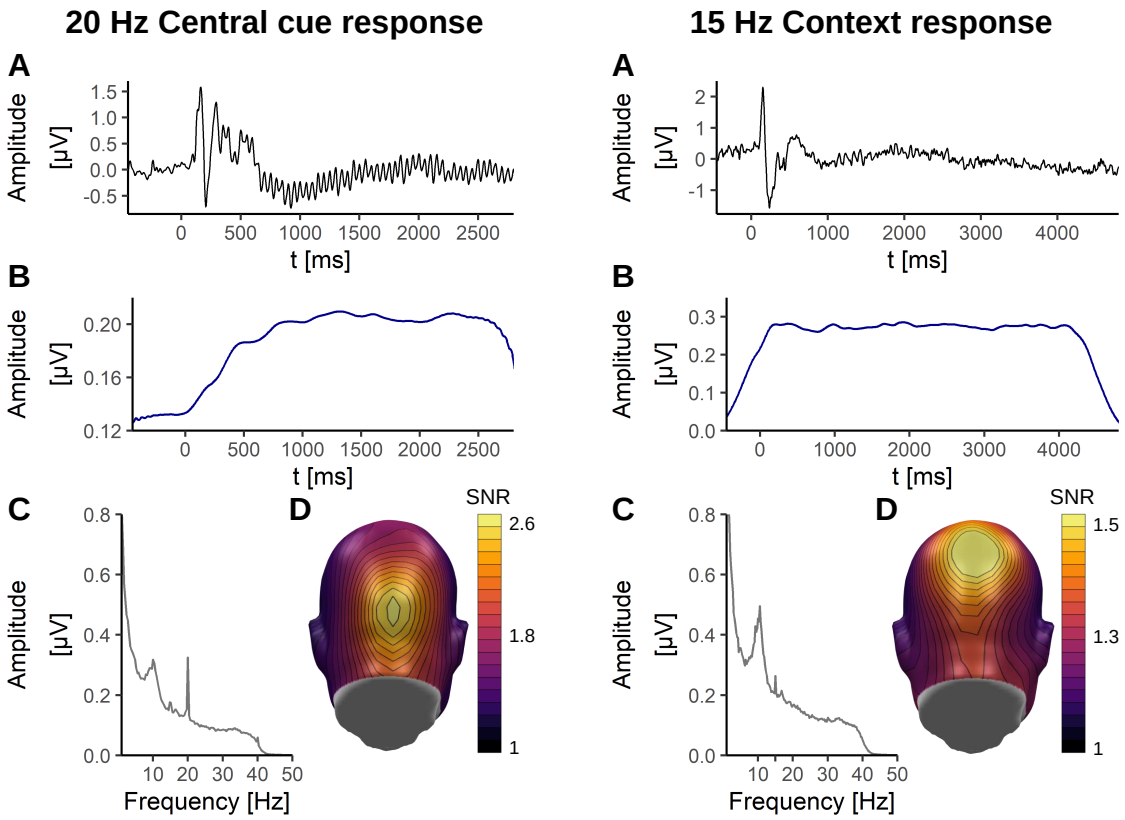

Figure 2.2: Characteristics of the grand averaged ssVEP signal during central and context cue presentations across all participants and conditions at Oz (sensor 75): (A) Time-domain representation of the raw ssVEP response. (B) Time-frequency analysis of the Hilbert-transformed driving frequencies. (C) Frequency-domain representation. (D) Topographies of the signal-to-noise ratio.

2.2.4 EEG recording and data analysis

EEG was recorded using a 129 electrodes Electrical Geodesics System (EGI, Eugene, OR) with a sampling rate of 250 Hz and an on-line band-pass filter of 0.1-100 Hz. The recording signal was referenced to the vertex electrode (Cz) and electrode impedances were kept below 50 kΩ. Using the EMEGS software for Matlab (Peyk, De Cesarei, & Junghofer, 2011), all data were filtered with a 40-Hz low-pass filter (cut-off at 3 dB point; 45 dB/octave, 19th order Butterworth), before extracting segments from 600 ms pre- to 4,900 ms post-onset for context and 600 ms pre- to 2,900 ms post-

onset for central cue responses. Channel and global artefacts were identified and replaced, following the guidelines for the statistical correction of artefacts in dense array studies procedure (SCADS; Junghofer, Elbert, Tucker, & Rockstroh, 2000). Trials were removed, if they included more than 20 contaminated sensors. After rejection, bad sensors of the remaining epochs were replaced by interpolation, using weighted spherical splines fit to all remaining sensors. The rejection rate was 28 $\pm$ 7 % ($M \pm SD$) for context and 23 $\pm$ 10 % ($M \pm SD$) for central cue responses. Remaining epochs were averaged separately for the two main phases and the three context conditions. The averaged epochs were analyzed in the time-domain using the Hilbert transform: First, the data were submitted to a bandpass-filter (width 0.5 Hz, 12th order Butterworth) around the driving frequencies of 15 Hz for context or 20 Hz for central cue presentations. The time-varying amplitude of the ssVEP signal was then extracted as the modulus of the filtered empirical signal and the Hilbert-transformed analytic signal. The raw ssVEP signals during central cue and context presentations for a representative electrode (Oz), the Fast-Fourier-Transformation on these ssVEPs, the time-frequency representations of the driving frequencies, and the topography of their SNRs averaged across all subjects and conditions are shown in Fig. 2.2. For statistical analyses, the ssVEP amplitudes were averaged across time points (between 100 ms to 2,900 ms for central cue and between 100 ms to 4,900 ms for context onset) and sensors (Oz and its 6 surrounding sensors for central cue and Oz and its 20 surrounding sensors for context analysis).

2.2.5 Statistical Analysis

Mean ssVEP amplitudes to central and context cues and ratings were analyzed separately for acquisition and extinction, using analysis of variances (ANOVA) with the within-subject factor condition (3 levels: N vs U vs P) and the between-subjects factor anxiety group (2 levels: LA vs HA). A significance level of 0.05 was used for all analy-

ses and Greenhouse-Geisser correction was applied where appropriate (Greenhouse & Geisser, 1959). Throughout this manuscript, corrected degrees of freedom, corrected p values and the partial η^2 ($\eta_p{}^2$) or Cohen's d (d) for follow-up t-tests and their 95% confidence interval are reported (Picton et al., 2000). In order to further explore the correlation between individual anxiety and attentional processing, bivariate Pearson correlations were calculated for STAI-Trait scores and difference ssVEP-amplitudes between P- and N-condition for acute and U- and N-condition for potential threat responding (Gorka et al., 2017b). Since participants were divided into extreme groups, correlations were separately analyzed for low and high anxious participants.

2.3 Results

2.3.1 Central cue analysis

Steady-state visual evoked potentials

During acquisition, there was a significant main effect of condition for ssVEP amplitudes in response to the central cues, $F(1.70, 95.46) = 3.48$, $p = .042$, $\eta_p^2 = .06$ $[0.00; 0.14]$, without any effect of group, $ps > 0.897$ (see Fig. 2.4 and Fig. 2.3). Follow-up t-tests showed higher ssVEP responses to the P- compared to the U-condition, $t(57) = 2.29$, $p = .026$, $d = 0.30$ $[0.04; 0.56]$, but no differences were found between the P- and N-condition, $t(57) = -1.49$, $p = .143$, $d = -0.20$ $[-0.45; 0.07]$, or the U- and N-condition, $t(57) = 1.45$, $p = .153$, $d = 0.19$ $[-0.07; 0.45]$. During extinction, no effect reached significance, $ps > 0.158$.

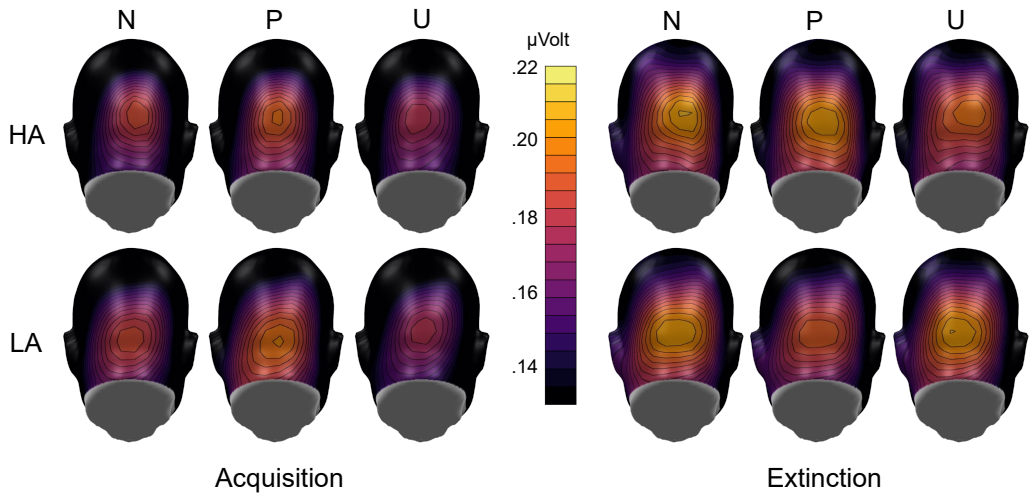

Figure 2.3: Topographies of the mean ssVEP amplitudes to the central cues during the acquisition phase (left) and the extinction phase (right) of the NPU threat test. High anxious individuals are shown in the upper rows, low anxious individuals in the bottom rows

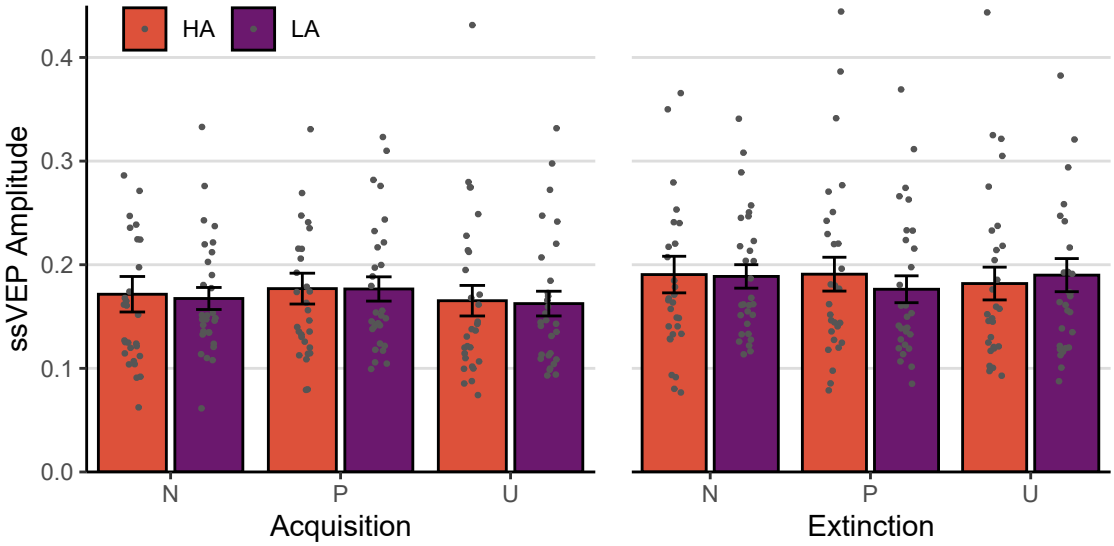

Figure 2.4: Mean ssVEP amplitudes (±SEM) to the central cues during the acquisition phase (left) and the extinction phase (right) of the NPU threat test.

Ratings of central cues with contexts

Threat ratings showed successful differential learning for the combinations of contexts and central cues (see Fig. 2.5). During acquisition, the ANOVA revealed a main effect

of condition, $F(2, 112) = 154.71$, $p < .001$, $\eta_p^2 = .73$ [0.66; 0.78], while there were no effects for group, $ps > 0.208$. As expected, follow-up t-tests demonstrated higher threat ratings for the P-, $t(57) = -16.64$, $p < .001$, $d = -2.18$ [−2.66; −1.71], and the U-condition, $t(57) = -11.58$, $p < .001$, $d = -1.52$ [−1.90; −1.14], compared to the N-condition. In addition, cues in the P-condition were rated as more threatening than cues in the U-condition, $t(57) = 4.79$, $p < .001$, $d = 0.63$ [0.34; 0.91]. These differences remained significant during extinction, $F(1.61, 90.23) = 42.81$, $p < .001$, $\eta_p^2 = .43$ [0.30; 0.53], N vs P: $t(57) = -7.53$, $p < .001$, $d = -0.99$ [−1.30; −0.67], N vs U: $t(57) = -6.77$, $p < .001$, $d = -0.89$ [−1.19; −0.58], P vs U: $t(57) = 2.02$, $p = .048$, $d = 0.27$ [< 0.01; 0.53], without any effect of group, $ps > 0.241$.

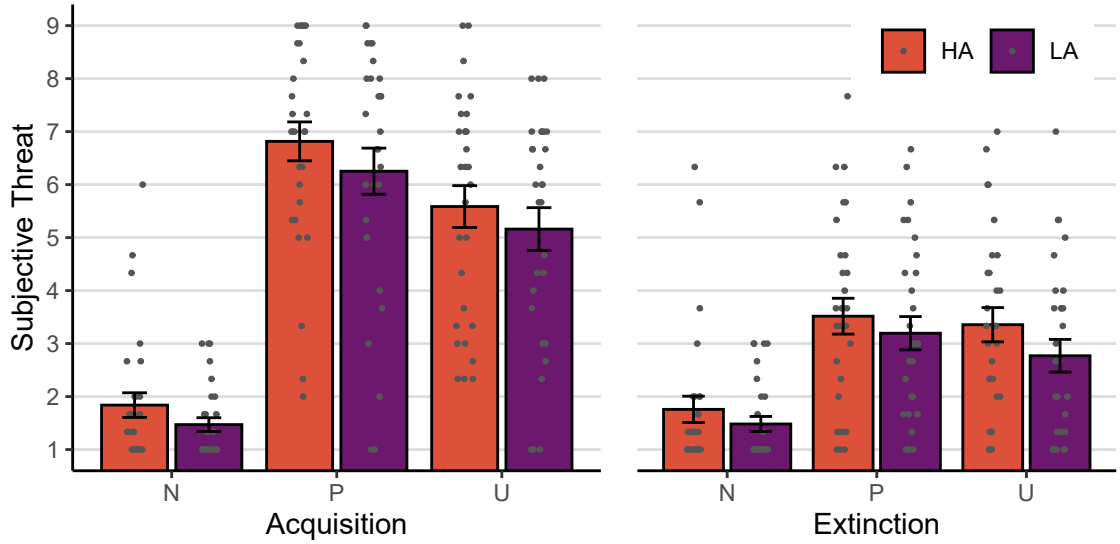

Figure 2.5: Mean threat ratings (±SEM) to the combination of central cues and contexts during the acquisition phase (left) and the extinction phase (right) of the NPU threat test.

US-contingency ratings during acquisition yielded a similar pattern, showing a significant main effect of condition, $F(1.62, 90.60) = 283.22$, $p < .001$, $\eta_p^2 = .83$ [0.78; 0.87], without any effects of group, $ps > 0.463$ (see Fig. 2.6). Follow-up t-tests revealed that cues in the P-, $t(57) = -30.56$, $p < .001$, $d = -4.01$ [−4.79; −3.23] and in the U-condition, $t(57) = -11.93$, $p < .001$, $d = -1.57$ [−1.95; −1.18], were

associated with higher US-contingency than in the N-condition. While contingency ratings were also higher for cues in the P- compared to the U-condition, $t(57) = 9.75$, $p < .001$, $d = 1.28$ [0.93; 1.63]. The differences between conditions stayed significant during extinction, $F(1.50, 83.88) = 34.94$, $p < .001$, $\eta_p^2 = .38$ [0.24; 0.49], N vs P: $t(57) = -6.63$, $p < .001$, $d = -0.87$ [−1.17; −0.56], N vs U: $t(57) = -11.93$, $p < .001$, $d = -1.57$ [−1.95; −1.18], P vs U: $t(57) = 9.75$, $p < .001$, $d = 1.28$ [0.93; 1.63]. Again, no effects for group could be found, $ps > 0.359$.

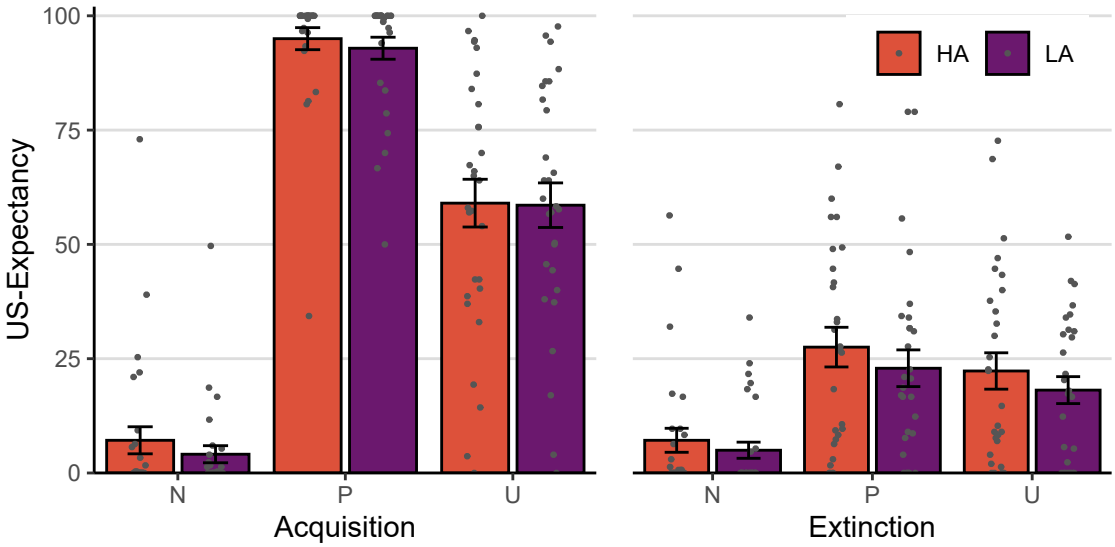

Figure 2.6: Mean US-expectancy ratings (±SEM) to the combination of central cues and contexts during the acquisition phase (left) and the extinction phase (right) of the NPU threat test.

2.3.2 Context analysis

Steady-state visual evoked potentials

During acquisition, ssVEP amplitudes to the contexts revealed a main effect of condition, $F(2, 112) = 3.57$, $p = .032$, $\eta_p^2 = .06$ [0.00; 0.13], which was further qualified by a significant Group x Condition interaction, $F(2, 112) = 4.23$, $p = .017$, $\eta_p^2 = .07$ (see Fig. 2.8 and Fig. 2.7). The main effect of group was not significant, $F(1, 56) = 2.07$, $p = .156$, $\eta_p^2 = .04$ [0.00; 0.14]. To follow-up on the interaction,

separate ANOVAs were calculated for the groups. The analysis for the low anxious group yielded a significant main effect of condition, $F(2, 56) = 5.99$, $p = .004$, $\eta_p^2 = .18$ [0.04; 0.30]. In contrast, there was no effect for the high anxious group, $F(2, 56) = 0.82$, $p = .446$, $\eta_p^2 = .03$ [0.00; 0.11]. For low anxious participants, the P-, $t(28) = -3.03$, $p = .005$, $d = -0.56$ [−0.95; −0.17], and the U-, $t(28) = -2.95$, $p = .006$, $d = -0.55$ [−0.93; −0.15], context elicited stronger ssVEP amplitudes than the N context, whereas no difference could be found between the P- and U-context, $t(28) = 0.37$, $p = .713$, $d = 0.07$ [−0.30; 0.43]. In addition, direct group comparisons revealed larger ssVEP amplitudes in response to the U-context for low compared to high anxious individuals, $t(28) = -2.40$, $p = .023$, $d = -0.45$ [−0.82; −0.06]. During extinction, there was a main effect of condition, $F(2, 112) = 3.87$, $p = .024$, $\eta_p^2 = .06$ [0.00; 0.14], but no effect of group, $ps > 0.149$. Visuocortical responses were enhanced for the N- compared to the P- and U-context, $t(57) = 2.27$, $p = .027$, $d = 0.30$ [0.03; 0.56] and $t(57) = 2.60$, $p = .012$, $d = 0.34$ [0.08; 0.61], while no difference was found between the U- and P- context, $t(57) = 0.30$, $p = .763$, $d = 0.04$ [−0.22; 0.30].

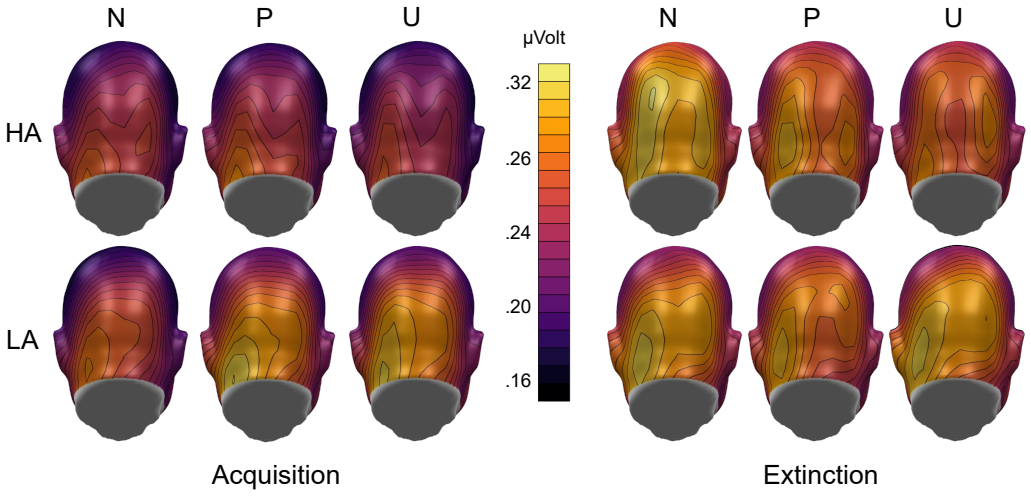

Figure 2.7: Topographies of the mean ssVEP amplitudes to the contexts during the acquisition phase (left) and the extinction phase (right) of the NPU threat test. High anxious individuals are shown in the upper rows, low anxious individuals in the bottom rows

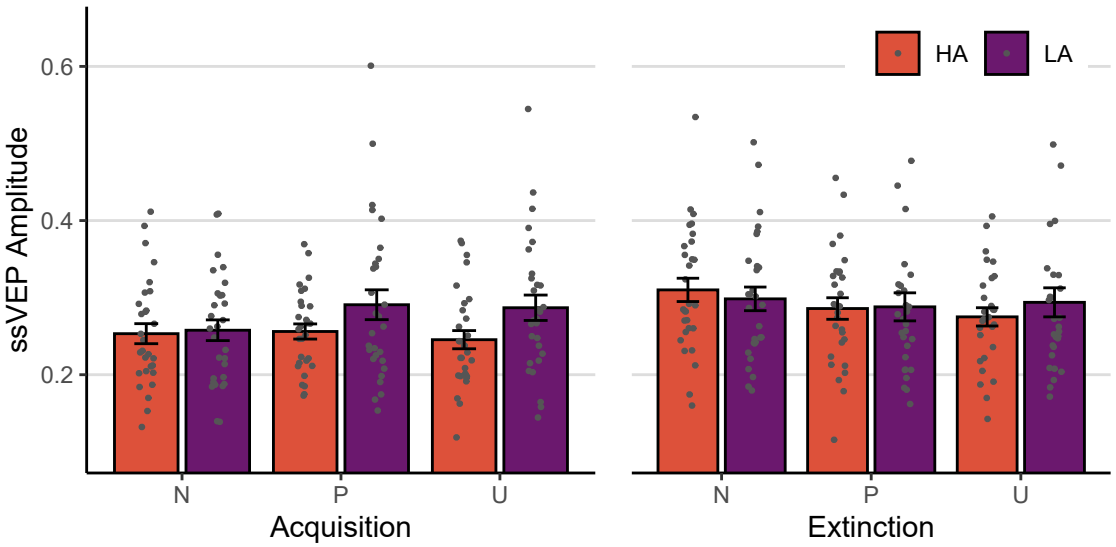

Figure 2.8: Mean ssVEP amplitudes (±SEM) to the contexts during the acquisition phase (left) and the extinction phase (right) of the NPU threat test.

Context ratings

During acquisition, threat ratings of the contexts revealed a significant main effect of condition, $F(2, 112) = 134.79$, $p < .001$, $\eta_p^2 = .71$ [0.63; 0.76], and a marginal effect of group, $F(1, 56) = 3.84$, $p = .055$, $\eta_p^2 = .06$ [0.00; 0.18]. However, there was no Group x Condition interaction, $F(2, 112) = 0.95$, $p = .390$, $\eta_p^2 = .02$ [0.00; 0.06] (see Fig. 2.9). High anxious individuals rated all contexts as more threatening than low anxious individuals. Follow-up t-tests for the main effect of condition showed higher threat ratings for the U- compared to the N- and P-context, $t(57) = -18.14$, $p < .001$, $d = -2.38$ [−2.88; −1.87] and $t(57) = -7.34$, $p < .001$, $d = -0.96$ [−1.27; −0.65]. The P-context was also rated as more threatening than the N-context, $t(57) = -8.41$, $p < .001$, $d = -1.10$ [−1.43; −0.77]. During extinction, these differences stayed significant, $F(2, 112) = 35.76$, $p < .001$, $\eta_p^2 = .39$ [0.27; 0.48], P vs N: $t(57) = -5.05$, $p < .001$, $d = -0.66$ [−0.95; −0.38], U vs N: $t(57) = -7.96$, $p < .001$, $d = -1.05$ [−1.36; −0.72], P vs U: $t(57) = -3.36$, $p = .001$, $d = -0.44$ [−0.71; −0.17]. In addition, the ANOVA showed a marginal main effect of group, $F(1, 56) = 2.99$, $p =$

.089, $\eta_p^2 = .05$ [0.00; 0.17], and a marginal interaction between group and condition, $F(2, 112) = 2.44$, $p = .092$, $\eta_p^2 = .04$ [0.00; 0.11]. Exploratory group comparisons yielded higher threat ratings to the U-context for high compared to low anxious individuals, $t(28) = 2.11$, $p = .044$, $d = 0.39$ [0.01; 0.77].

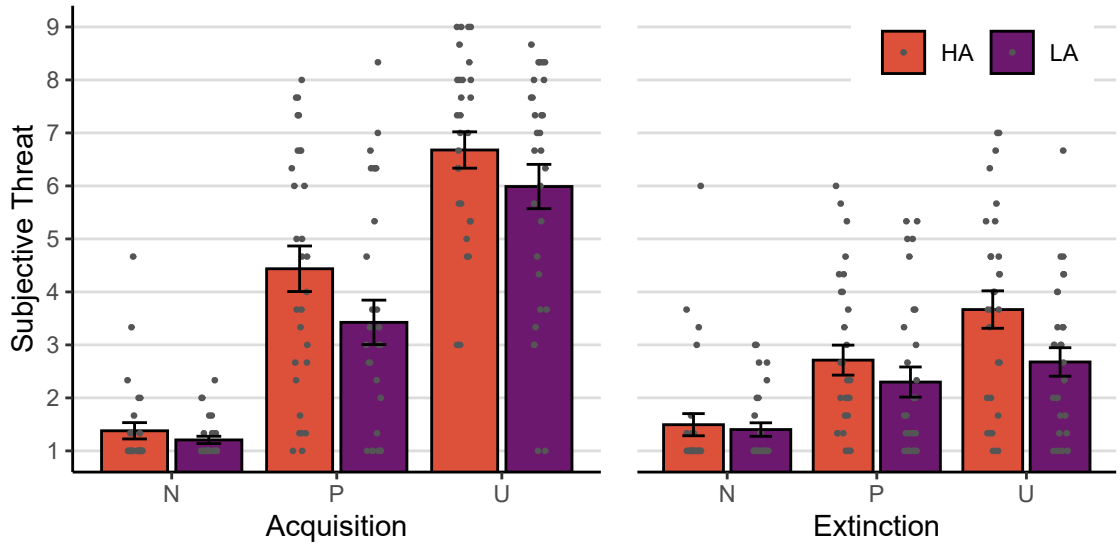

Figure 2.9: Mean threat ratings (±SEM) to the contexts during the acquisition phase (left) and the extinction phase (right) of the NPU threat test.

Analysis of the US-contingency ratings during acquisition yielded a main effect of condition, $F(1.50, 84.01) = 154.49$, $p < .001$, $\eta_p^2 = .73$ [0.65; 0.78], and a main effect of group, $F(1, 56) = 4.95$, $p = .030$, $\eta_p^2 = .08$ [0.00; 0.21], without an interaction, $F(1.50, 84.01) = 1.05$, $p = .338$, $\eta_p^2 = .02$ [0.00; 0.08] (see Fig. 2.10). Follow-up t-tests showed significant differences among all conditions, U > N: $t(57) = -25.90$, $p < .001$, $d = -3.40$ [−4.07; −2.72], U > P: $t(57) = -9.13$, $p < .001$, $d = -1.20$ [−1.53; −0.86], P > N: $t(57) = -6.09$, $p < .001$, $d = -0.80$ [−1.09; −0.50], with generally higher US-contingency ratings for high anxious individuals. During extinction, these effects stayed significant, $F(2, 112) = 24.05$, $p < .001$, $\eta_p^2 = .30$ [0.18; 0.40], U > N: $t(57) = -7.07$, $p < .001$, $d = -0.93$ [−1.23; −0.62], U > P: $t(57) = -2.59$, $p = .012$, $d = -0.34$ [−0.60; −0.07], P > N: $t(57) = -4.28$, $p < .001$, $d = -0.56$ [−0.84; −0.28], without

any effect of group, $ps > 0.408$.

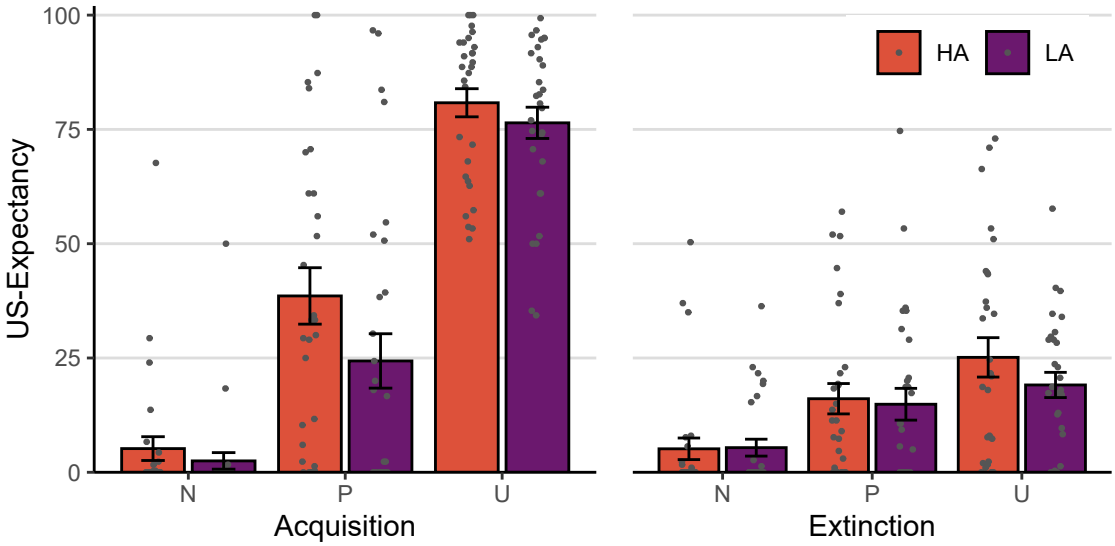

Figure 2.10: Mean US-Expectancy ratings (±SEM) to the contexts during the acquisition phase (left) and the extinction phase (right) of the NPU threat test.

2.3.3 Cue-locked context analysis

Mean visuocortical responses to the central-cue-locked context-cues revealed neither a main effect of condition, $F(2, 112) = 0.65$, $p = .526$, $\eta_p^2 = .01$ [0.00; 0.05], nor a main effect of group, $F(1, 56) = 1.48$, $p = .229$, $\eta_p^2 = .03$ [0.00; 0.12], nor their interaction, $F(2, 112) = 0.19$, $p = .830$, $\eta_p^2 < .01$ [0.00; 0.03], indicating no differential responses to the contexts when central cues were presented (see Fig. 2.11).

Similar results were obtained for the extinction phase (see Fig. 2.11), indicating no effect of condition, $F(2, 112) = 0.84$, $p = .433$, $\eta_p^2 = .01$ [0.00; 0.06], group, $F(1, 56) = 0.22$, $p = .641$, $\eta_p^2 < .01$ [0.00; 0.07], and no significant Condition x Group interaction, $F(2, 112) = 0.14$, $p = .866$, $\eta_p^2 < .01$ [0.00; 0.02].

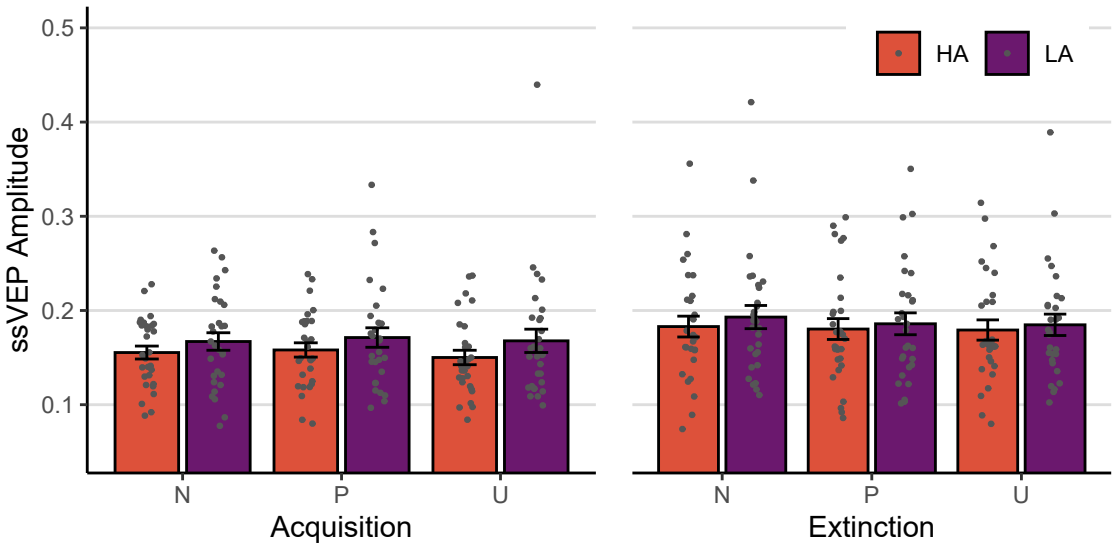

Figure 2.11: Mean ssVEP amplitudes (±SEM) to the cue-locked context presentations during the acquisition phase (left) and the extinction phase (right) of the NPU threat test.

2.3.4 Correlational analysis

Correlation analyses between STAI-Trait and ssVEP amplitudes within groups revealed a significant correlation between STAI trait scores and potential threat responding (see Fig. 2.12) for high but not for low anxious individuals. Higher STAI trait scores were associated with reduced ssVEP amplitudes to contexts during the U- compared to the N-condition, $r(27) = -.53$, $p = .003$. This correlation did not reach significance for low anxious individuals, $r(27) = .17$, $p = .391$. No significant correlations were observed for acute threat (high anxious: $r(27) = -.06$, $p = .763$; low anxious: $r(27) = .10$, $p = .596$).

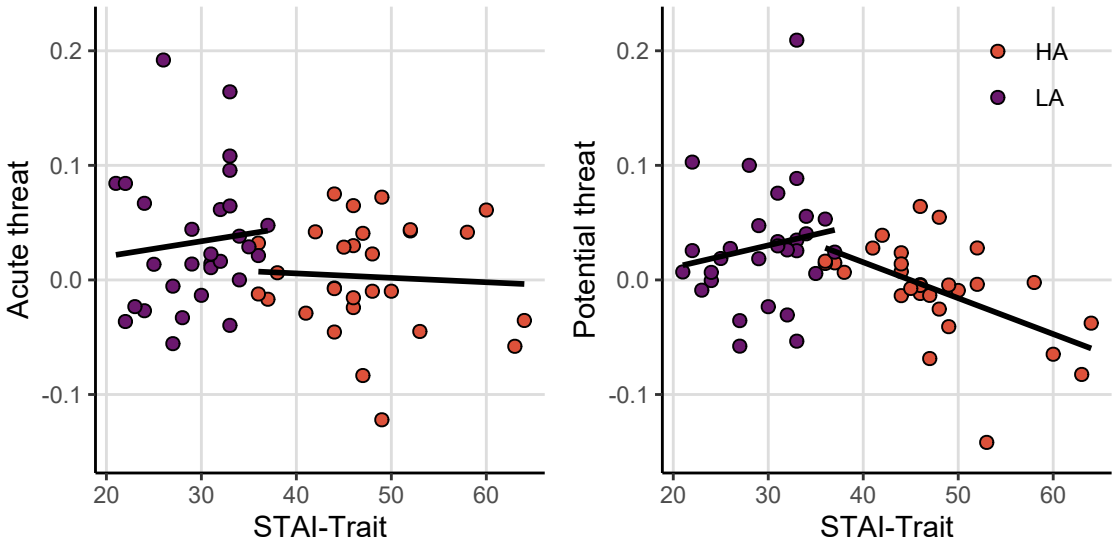

Figure 2.12: Scatterplots for individual STAI-Trait scores and ssVEP amplitudes. Acute threat (left): Difference of central cue elicited ssVEP-amplitudes between P- and N-condition; Potential threat (right): Difference of context elicited ssVEP amplitudes between U- and N-condition. In both figures, group allocation is illustrated by color.

2.4 Discussion

The purpose of the present study was threefold: The first goal was to examine the electrocortical correlates of attention during acute and potential threat. The second goal was to compare high vs. low anxious individuals regarding attention allocation. The final goal was to test the stability of these processes during extinction learning. To achieve these goals, this study utilized steady-state visual evoked potentials along with ratings of perceived threat and US-expectancy in a combined cue and context conditioning paradigm.

Threat and US-expectancy ratings revealed successful induction of predictable and unpredictable threat: Both groups showed elevated ratings to the contexts in the unpredictable threat condition and to the combination of context and central cue in the predictable threat condition, further substantiating earlier results (Wieser et al.,

2016b). Moreover, ratings of threat and US-expectancy diminished during extinction.

There were no differences between low and high anxious individuals regarding threat and US-expectancy ratings to the central cues. However, high anxious individuals rated the contexts generally as more threatening and more associated with an US than low anxious participants. These results show enhanced sensitivity to contextual threat in high anxious individuals, which parallels a vast body of literature suggesting that anxiety disorders are characterized by heightened sensitivity to potential threat (Gorka et al., 2017b; Grillon et al., 2008, 2009) and extend these findings to participants at risk from these disorders. In addition, follow-up analyses during extinction learning yielded higher threat ratings of unpredictable threat in high anxious compared to low anxious individuals. This result is in line with a recent meta-analysis, indicating reduced extinction learning in anxiety patients (Duits et al., 2015; Lissek et al., 2005). Please note that these meta-analytical results are primarily based on fear conditioning paradigms, while in the present experiment, high trait-anxious individuals showed reduced extinction during the unpredictable threat condition only. Yet, this finding lends further evidence for the notion of enhanced sensitivity to potential threat in high anxious individuals.

Regarding visuocortical activity during acute and potential threat, ssVEP amplitudes to the central cue were enhanced in the P- compared to the U-condition. This result indicates facilitated threat-related sensory processing and demonstrates further evidence for the electrocortical correlate of selective attention to acute threat (Kastner-Dorn et al., 2018; Miskovic & Keil, 2012; Richards et al., 2014).

In contrast, visuocortical engagement to contexts revealed different patterns for high and low anxious individuals. While low anxious individuals showed increased ssVEP amplitudes to the U- and P-contexts compared to the N-contexts, high anxious individuals did not differentiate between conditions. The results for low anxious individuals parallel findings of Kastner-Dorn et al. (2018), who also investigated

ssVEPs during a NPU-threat task. The authors discussed that contexts associated with aversive events facilitate sensory processing compared to completely safe contexts. Crucially, in the present study, the onset of the predictable as well as the unpredictable context signal aversive events during the upcoming block. In the U-condition, US are administered independent of the central cue, making the context the next best predictor. During the predictable context, US are further signaled by a discrete cue. The onsets of these discrete cues, however, are also unpredictable, potentially leading to a sustained state of anxious apprehension. Consequently, enhanced visuocortical activity to the P- and the U-context represent electrocortical correlates of hypervigilance during potential threat.

Contrary to our expectations, present results yielded no differential visuocortical responding to the contexts for high anxious individuals. Yet, a similar response pattern was observed in a recent study by Ahrens et al. (2015). In this study, different neutral faces were either associated with neutral, positive, or negative verbal comments. The authors used ssVEPs to measure attention allocation to the conditioned facial stimuli in high and low social anxious individuals. Results revealed that high social anxious individuals in contrast to low social anxious individuals did not differentiate between the three conditions regarding ssVEP amplitudes, hinting at an impaired ability to discriminate between safety- and threat-related social stimuli. Since high social anxious individuals compared to low social anxious individuals also demonstrated generally enhanced ssVEP amplitudes to the facial identities, the authors suggested a potential hyperactivation of the amygdala in high social anxious individuals, consequently leading to an overgeneralization of conditioned social threat. In the present study, however, high anxious individuals showed reduced ssVEP amplitudes to the threat-related contexts compared to low anxious individuals. Accordingly, these results suggest a potential indicator of perceptual avoidance, instead of overgeneralization of potential threat in early stages of visuo-

cortical processing. Even though attentional hypervigilance-avoidance biases have been discussed in anxiety (Onnis, Dadds, & Bryant, 2011), empirical evidence for avoidance in ssVEP measures is scarce (Wieser et al., 2011). First evidence stems from a recent study, which observed diminished visuocortical engagement to aversive compared to neutral facial expressions for social anxiety patients (McTeague et al., 2018). In this study, ssVEP responding to aversive stimuli depended on the severity of the anxiety disorder: patients with mild social anxiety showed increased responding to aversive facial expressions, while ssVEP amplitudes of the most severely impaired patients were actually reduced, potentially reflecting a visuocortical correlate of perceptual avoidance (McTeague et al., 2018). Even though in the present study only a high anxious analog sample was investigated, these results indicate initial hypervigilance and consequent perceptual avoidance of aversive stimuli as a potential risk factor for high anxious individuals (Mogg & Bradley, 1998). Crucially, it should be noted that the initial short-lasting hypervigilance might be difficult to capture due to early non-stationary components of the ssVEP (Miskovic & Keil, 2013b, 2013a). Furthermore, correlational analyses yielded a negative association for high anxious individuals between STAI-trait scores and differential processing of the contexts during unpredictable compared to neutral condition. The more anxious individuals were the more they perceptually avoided the unpredictable threat context. This sensory processing bias could also explain the elevated threat and contingency ratings for the contexts independent of the conditions, since high anxious individuals might be worse at discriminating context threat stimuli and accordingly overestimate the impact of unpredictable threat.

In contrast to the findings by Wieser et al. (2016b), the present study yielded no differences in visuocortical activity during central cue-locked analysis of the contexts between the conditions. The original study found facilitated sensory processing of the contexts during presentations of the central cue in the predictable threat condition.

It is important to mention, however, that the authors used the same central cue for all conditions and, thus, the central cue in the predictable condition only had signaling value in combination with the contexts. Wieser and colleagues suggested that participants might have widened their attentional focus to identify the current condition. In the present study, different central cues were used for each condition. Consequently, participants did not need to rely on the simultaneously presented contexts to identify in which condition they were.

During extinction learning, only the context which formerly indicated safety gained attentional resources. This pattern is well known in the ssVEP fear conditioning literature (Keil, Miskovic, Gray, & Martinovic, 2013; McTeague et al., 2015) and can be explained by basic computational models (Yuan et al., 2018). Accordingly, increased visuocortical processing of the former non-predictive stimuli may reflect the visuocortical correlate of extinction or represent attentional reorientation processes as participants expected this condition to be associated with aversive events during extinction.

Since later time intervals of the context presentation were confounded with electrical stimulations, which may have led to artifacts in the EEG, the current study was limited to analyses of the onset of the contexts. Therefore, the current study is not able to make statements about visuocortical responding to the contexts during the whole block. Even so there is some evidence that threat-related attention is stable over time (Kastner et al., 2015; Kastner-Dorn et al., 2018), it is important to consider extinction processes as well as variations between healthy and pathological forms of anxiety throughout the whole context presentations in future studies.

Altogether, the results of the present study lend further support for the use of ssVEPs to investigate the electrocortical correlates of selective attention and hypervigilance during acute and potential threat (Kastner-Dorn et al., 2018; Wieser et al., 2016b). In addition, individuals at risk for anxiety disorders seem to be characterized

by dysfunctional processing of unpredictable threat cues (Gorka et al., 2017b; Grillon et al., 2008) and an impaired ability to discriminate between aversive contexts, which could lead to perceptual avoidance of threatening relative to safe contexts on an early visuocortical level. During extinction learning, increased visuocortical responding to the safety stimuli may reflect electrocortical correlates of active extinction learning and perceptual reorientation processes after the change of contingencies (McTeague et al., 2015).

Finally, the NPU-threat task is well suited to investigate fear- and anxiety-like processes in the laboratory. While the central cue in the P-condition models acute threat, the whole U-context is associated with potential threat. Critically, in this task, acute and potential threat -and thus fear and anxiety- are strictly separated by the experimental conditions. There is growing evidence, however, that challenges this strict segregation of fear and anxiety (Fox & Shackman, 2019). In the present study, for example, threat ratings to the central cues in the U-condition were elevated, even though these cues actually signaled the absence of aversive events in the current design. There are several similar examples on how potential threat impacts the responding to actual threat and vice versa (e.g. Grillon, Franco-Chaves, Mateus, Ionescu, & Zarate, 2013; Kastner-Dorn et al., 2018). Therefore, it is necessary to consider interactions between threat processing mechanisms during acute and potential threat (Tovote et al., 2015). To address this issue, Study 2 examines potential interactions between fear and anxiety.

Study 2: Investigating attentional mechanisms in an orthogonal cue and context conditioning paradigm

3.1 Introduction

Over the last years, fear and anxiety has been established as two separate emotional states in the neuroscience literature (e.g. Davis et al., 2010). In brief, fear is a phasic response elicited by actual threat in the face of specific and imminent danger, while anxiety is a sustained feeling of apprehension linked to potential and diffuse threatening situations (Davis et al., 2010). Fear and anxiety have also been related to the different stages of the threat-imminence model, in which anxiety maps onto the pre-encounter stage, where threat might occur but has not yet been identified, whereas fear is triggered at the post-encounter stage after threat became imminent (Fanselow, 2018; Hamm, 2020; Lang et al., 2000; Mobbs et al., 2015). Regarding fear and anxiety, the threat-imminence model implies that either fear or anxiety is active at a given time and that there is a rapid transition from anxiety to fear once a threat has been detected. This seems to be the prevalent view in the human and animal literature (Davis et al., 2010; Sylvers et al., 2011; Tovote et al., 2015) and has even been integrated in the RDoC framework's negative valence systems as 'acute' and 'potential threat' (Cuthbert, 2014; Insel, 2014; and Insel et al., 2010).

Evidence for a separation of fear and anxiety primarily results from pharmacologi-

cal and lesion studies in rodents. These studies usually target the extended amygdala, which includes the bed nucleus of the stria terminalis (BNST) next to the amygdaloid nuclei (Davis et al., 2010). There is convincing proof that acute threat prompts central amygdala (CeA) activity, while potential threat is associated with enhanced activity in the BNST (Davis, 1992; Davis, Walker, & Lee, 1997; Davis et al., 2010; Tovote et al., 2015). These results have led to the notion that the CeA is the key hub in the neural fear network, while the BNST takes an equal role in the neural circuit underlying anxiety (e.g. Tovote et al., 2015). Crucially, it could be demonstrated that lesions of the CeA, but not the BNST, block responding to acute threat in a fear conditioning paradigm (Hitchcock & Davis, 1991). On the other hand, neurotoxic lesions of the BNST, but not the CeA, reduce anxiety-like responses (light-enhanced startle reflexes), while keeping fear-potentiated startle intact (Walker & Davis, 1997). Similarly, BNST deactivation blocks defensive responding to conditioned cues that were presented for a sustained, but not short period, while CeA deactivation does not impact responses to sustained, but short cues (for a review see Davis et al., 2010). Taken together, these findings substantiate the idea of a dissociation between the CeA and BNST in their role for fear and anxiety.

Translating these results to the human brain, Herrmann et al. (2016) showed that amygdala activity was enhanced during the onset of an aversive conditioned cue, while sustained presentations of the same cue prompted BNST activity instead. An adapted version of the NPU-threat task for fMRI revealed elevated BNST activity during the unpredictable threat compared to the neutral context, whereas amygdala responses were elevated to the discrete cue in the predictable threat compared to the neutral condition (Alvarez et al., 2011). In addition, Somerville et al. (2013) used a mixed-block-paradigm, in which they briefly presented aversive pictures either in a predictable context (signaled by a countdown) or in an unpredictable context to investigate transient and sustained neural responses. Measuring BOLD-responses, they

found enhanced transient responses in the amygdala to negative compared to neutral pictures, whereas unpredictable versus predictable contexts increased sustained activity in the BNST, indicating unique neural systems for fear- and anxiety-like responses. Most importantly, however, since the authors presented aversive and neutral pictures either in predictable or unpredictable blocks, they could examine a potential interaction between sustained and transient responses. Interaction analysis indeed demonstrated potentiated transient amygdala responses to aversive pictures in unpredictable compared to predictable contexts. Crucially, this potentiation was not evident for neutral pictures, suggesting that sustained anxiety-like responses might facilitate transient fear-like responses.

Moreover, there is growing evidence from human fMRI research being incompatible with a strict-segregation model of the CeA and BNST for fear and anxiety (for a review, see Fox & Shackman, 2019). For example, Andreatta et al. (2015) utilized a context conditioning paradigm in virtual reality to investigate neural activity during potential threat. In contrast to the studies mentioned above, the authors found amygdala and hippocampus activity during sustained presentations of the anxiety context, while initial activity to the context onsets was mainly found in prefrontal cortices. Equally, evidence from two different potential threat tasks convergently demonstrated enhanced amygdala, but no enhanced BNST activity during potential threat (Gorka et al., 2017a). Instead, potential threat also elicited sustained BOLD responses in the hippocampus, insula and prefrontal regions. Further proof stems from a study with rats, which were selectively bred for high and low context conditioned freezing using a short term selective breeding strategy. Crucially, the high anxiety animals also demonstrated potentiated startle responses to transient CS (Ponder et al., 2007), suggesting that the systems for fear and anxiety are probably not independent from each other and may interact at some level.

There are two main reasons for these inconsistencies in the literature: First, until

now there has been no mechanistic account for the interaction of fear and anxiety, which is essential for the generation of testable hypotheses. And second, suitable paradigms to investigate the potential interaction between fear and anxiety are still lacking. As mentioned above, the NPU-threat task has previously been used to compare responding during acute and potential threat (Schmitz & Grillon, 2012). In this task, however, acute and potential threat are strictly separated by the experimental conditions, thus, dismissing potential interactions. To test hypotheses regarding the interaction of fear and anxiety, the present study utilized a novel paradigm, in which fear conditioning is realized within a context conditioning paradigm to orthogonally induce acute and potential threat. I expected elevated transient responses to the fear compared to a neutral cue and elevated sustained responses to the anxiety compared to a neutral context. Regarding the interaction of cue and context conditioning, I expected potentiated responses to the fear cue during the anxiety compared to the neutral context. To quantify threat detection and defensive responding, this study leverages the benefits of steady-state visual evoked potentials (ssVEPs) as an index of visuocortical activity and skin conductance responses as a measure of psychophysiological arousal, along with behavioral ratings. Finally, the stability of these mechanisms is examined during an additional extinction phase.

3.2 Methods and materials

All methods and analyses have been preregistered at the Open Science Framework (`https://osf.io/gcjft`).

3.2.1 Sample

Forty-seven subjects participated in the experiment, of which two had to be excluded because of data recording failure. The final sample therefore consisted of 45 partici-

pants (36 females, mean age ± SD: 23.51 ± 3.31 years). Participants were required to be between 18 and 35 years old and excluded from the experiment if they reported any actual mental or neurological disorder or any family history of photic epilepsy. At the beginning of the study, all participants gave written informed consent and completed the German versions of Spielberger's State-And-Trait Anxiety Inventory (STAI; Laux & Spielberger, 1981), the Anxiety Sensitivity Index 3 (ASI-3; Reiss et al., 1986; Taylor et al., 2007), the Intolerance of Uncertainty Scale (IUS; Buhr & Dugas, 2002), the GAD-7 questionnaire (Spitzer et al., 2006), and the Depression-Anxiety-Stress-Scale (DASS; Lovibond & Lovibond, 1995). For the descriptive statistics of the questionnaires see Table 3.1. Participants were paid 12 € or received an equivalent in course credits as reimbursement. All procedures were approved by the ethics committee of the University of Würzburg.

3.2.2 Stimuli and Apparatus

Two differently oriented (0° and 90°) Gabor patches, consisting of sinusoidal grating (Gaussian-windowed with maximal contrast at center) with a spatial frequency of 1.4 cycles per degree, indexed the CS+ and CS- for aversive cue conditioning. The contexts, indicating the CTX+ and CTX-, consisted of four hexagons or dodecagons, presented peripherally in the corners of the monitor ca. 3.76° of visual angle from the central Gabor patch, spanning visual angles of 1.87°. The Gabor patches spanned visual angles of 2.83°. To enable a disentangling of the visuocortical responses (ssVEPs) evoked by central and context cues, central cues were presented in 20 Hz flickering mode, while contexts were presented in 15 Hz flickering mode. All visual stimuli were counter-balanced for conditions across participants. The stimuli were presented on a black background on a 19-inch monitor (resolution = 1024 x 768 pixels) with a vertical refresh rate of 60 Hz, located ca. 100 cm in front of the participant. Presentation of all stimuli was controlled using Presentation Software (Neurobehavioral Systems,

Inc., Albany, CA, USA).

Table 3.1: Sample characteristics and questionnaire data

	N	M	SD	Mdn	Min	Max
Age	45	23.51	3.31	23	19	34
STAI-S	45	34.40	5.07	34	26	45
STAI-T	45	36.18	6.86	36	22	59
ASI-3	45	17.56	9.35	16	0	37
IUS	45	42.09	8.89	42	25	58
GAD-7	45	4.11	2.54	4	0	11
DASS-D	45	2.69	2.43	2	0	11
DASS-A	45	1.62	1.76	2	0	8
DASS-S	45	4.84	3.77	4	0	14
US-Unpleasantness	45	6.56	1.56	7	3	10

Note: STAI = State-Trait-Anxiety-Inventory (S: Subscale State, T: Subscale Trait); ASI3 = Anxiety Sensitivity Index 3; IUS = Intolerance of Uncertainty Scale; DASS = Depression-Anxiety-Stress-Scale (D: Subscale Depression, A: Subscale Anxiety, S: Subscale Stress).

The unconditioned stimuli (US) were 95 dB white noise bursts presented binaurally via headphones (Sennheiser, Wedemark, Germany). Prior to the experiment, the US was presented once and participants rated the US aversiveness on a scale ranging from 0 (not unpleasant) to 10 (very unpleasant), resulting in a mean (± SD) aversiveness of 6.56 ± 1.56. There was no individual pain threshold calibration for the electro-tactile stimulus, since these calibration procedures usually provide participants with a sense of control over the US intensity and thereby reduce the anticipatory anxiety of the electro-tactile stimuli (Sperl, Panitz, Hermann, & Mueller, 2016).

3.2.3 Procedure

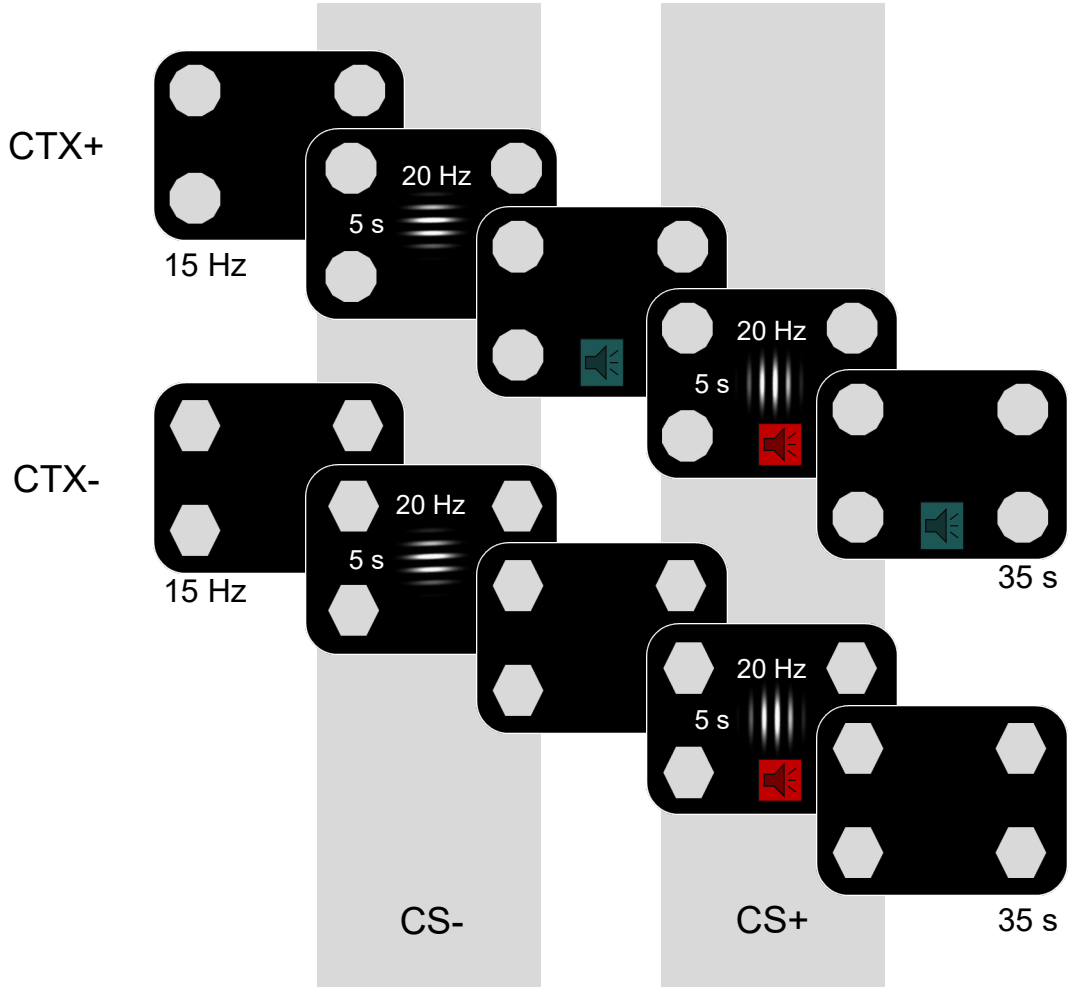

Figure 3.1: Experimental Design. The anxiety and neutral context conditions were indicated by geometrical symbols (hexagons or dodecagons), presented peripherally for 32 s in a flickering mode with a frequency of 15 Hz. During the context presentations, differently oriented (0° and 90°) grating stimuli were presented for 5 s with a flickering frequency of 20 Hz. In the acquisition phase, loud noise blasts were presented (US, reinforcement rate = 100%) at the offset of one of the grating stimuli (CS+). In addition, unpredictable US were presented at random time points during the anxiety context but not during the neutral context. US-administration was omitted during extinction.

After giving written informed consent, participants were seated in a sound-attenuated, dimly lit testing room, where physiological sensors and the EEG-net were applied. The study consisted of three phases: habituation, acquisition and

extinction phase. During habituation, each context condition was presented twice for 35 s. At random timepoints during this interval, the CS+ and CS- were presented once for 5 s (2 central cue presentations per context block). The ITI between two context blocks was 5 - 6 s and the ITI between two subsequent Gabor-patches was at least 8 s. Before the acquisition phase, participants were instructed about the US-contingencies. They were told that during the CTX+, loud, unpleasant noises would be presented at random timepoints, while no random noises would be presented during the CTX-. In addition, a loud, unpleasant noise would be presented at the end of the CS+ presentation, while there would be no noises during the CS- presentation. During acquisition (Fig. 3.1), both context conditions were presented 12 times. In each context block, two central stimuli were presented in random order (either two CS+, one CS+ and one CS- or two CS-), resulting in 12 CS+ and 12 CS- presentations during both contexts in total. US were delivered at the offset of a CS+ presentation (100% reinforcement rate) and in addition, one to three unpredictable US were presented during the CTX+ (24 unpredictable US in total). Importantly, no central stimulus or US (in CTX+) was presented during the first six seconds after context onset, as this interval was later used for analyses of the visuocortical responses to the contexts. The extinction phase was similar to the acquisition phase regarding the amount and timing of stimulus presentations, while US-delivery was omitted. Participants were not instructed about US-omission. Subjective levels of threat and US-expectancy were collected after habituation, acquisition and extinction and additionally after the first half of acquisition and extinction. Participants rated each central cue and context condition and every combination of central cue and context condition via visual analogue scales, ranging from 0 = not threatening/ not likely to 100 = very threatening/ very likely.

3.2.4 Physiological data processing

Skin conductance was recorded using two silver-silver chloride electrodes placed on the thenar and hypothenar eminences of the participants' left palmar surface. The signal was recorded with a V-Amp amplifier and Vision Recorder Software (BrainProducts Inc., Munich, Germany). A sampling rate of 1,000 Hz and a notch-filter at 50 Hz were applied. Analysis was then performed using Vision Analyzer Software (BrainProducts Inc., Munich, Germany). Trough-to-peak values within 1 s to 6 s after central cue onset were scored manually for each phase and condition (CS+ and CS- during fear acquisition and each combination of CS+ and CS- with CTX+ and CTX- during threat-of-shock phase). Individual responses smaller than 0.02 µS were scored as zero responses. All SCRs were square-root-transformed to account for eventual skewedness of the underlying data (Boucsein et al., 2012).

3.2.5 EEG recording and data processing

Electrocortical brain activity was recorded via 129 electrodes Electrical Geodesics System (EGI, Eugene, OR) referenced to the vertex electrode (Cz), with a sampling rate of 250 Hz and an on-line band-pass filter of 0.1-100 Hz. Electrode impedances for recording were kept below 50 kΩ. Subsequent data processing occurred off-line using the EMEGS software for Matlab. Firstly, all data were filtered using a 40-Hz low-pass filter (cut-off at 3 dB point; 45 dB/octave, 19th order Butterworth), before extracting epochs from 600 ms pre to 4,900 ms post cue-onset. Artifacts were identified and corrected according to the guidelines for the statistical correction of artifacts in dense array studies procedure (SCADS; Junghofer et al., 2000). The mean rejection rate was 25.2 ± 18.4 % ($M \pm SD$). Remaining epochs were averaged separately for the four combinations of cue and context conditions and the three experimental phases (habituation, acquisition, extinction). The current source densities (CSD) of the

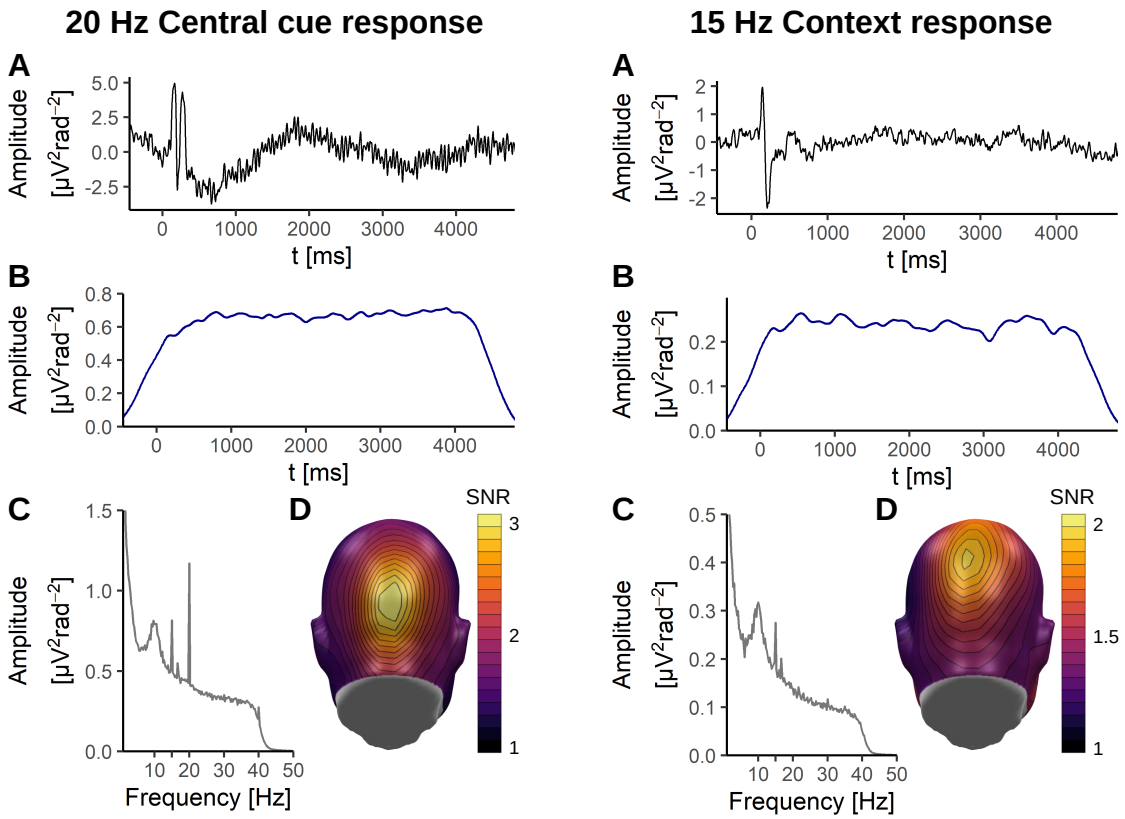

Figure 3.2: Characteristics of the grand averaged ssVEP signal during central cue and context presentations across all participants and conditions at Oz (sensor 75): (A) Time-domain representation of the CSD-transformed ssVEP response. (B) Time-frequency analysis of the Hilbert-transformed 20 Hz driving frequency. (C) Frequency-domain representation. (D) Topographies of the signal-to-noise ratio.

time-averaged data were calculated to account for volume conductance. The CSD approach employs a spatial Laplacian filter (the second spatial derivative) of the scalp potential to estimate the potential distribution at the cortical surface (Junghöfer, Elbert, Leiderer, Berg, & Rockstroh, 1997). The CSD transformed data were then transformed into the frequency domain using a Fast-Fourier-algorithm on a time interval between 1,000 and 4,600 ms post cue-onset. The first 1,000 ms after stimulus onset were omitted since effects of aversive conditioning on visuocortical responding are more prominent during a later stage of stimulus presentation due to early non-stationary components of the steady-state visual evoked potential (Miskovic & Keil,

2013b, 2013a). In a next step, we obtained the signal-to-noise ratios (SNR) for the driving frequency of the central cue presentations by dividing the power of the 20 Hz frequency by the mean of the spectral power at six adjacent frequency bins, leaving out the two immediate neighbors. This step was repeated for context analyses with the driving frequency of 15 Hz. The SNR is a unitless measure that accounts for both the evoked signal and the random noise in the data and has recently been used in other ssVEP paradigms as well (Barry-Anwar, Hadley, Conte, Keil, & Scott, 2018; Boylan et al., 2019). The CSD-transformed ssVEP signals for a representative electrode (Oz), the Fast-Fourier-Transformation on these ssVEPs, the time-frequency representations of the driving frequencies, and the topography of their SNRs averaged across all subjects and conditions are shown in Fig. 3.2. For statistical analyses, the ssVEP activity was pooled across Oz and 7 or 14 surrounding electrodes for central cue respectively context analyses (Wieser & Keil, 2014).

3.2.6 Statistical Analyses

Responses to the onset of the contexts and central cues were analyzed seperately. Mean differences in ratings, SCRs and ssVEPs to the context onsets were analyzed using ANOVAs with the within-subject factors context (CTX+ vs CTX-) and phase (acquisition vs extinction). While mean responses during the presentation of the central cue were analyzed seperately for each experimental phase using ANOVAs with the within-subject factors context (CTX+ vs CTX-) and cue condition (CS+ vs CS-). A significance level of 0.05 was used for all analyses and Greenhouse-Geisser correction was applied where appropriate. Throughout this manuscript, the corrected degrees of freedom, the corrected p values and the partial η^2 ($\eta_p{}^2$) or Cohen's d (d) and their 95% confidence interval are reported.

3.3 Results

3.3.1 Steady-state visual evoked potentials

Context responses

The ANOVA for the mean ssVEP amplitudes to the context onsets did not reveal any effect of context, $F(1, 46) = 2.12$, $p = .152$, $\eta_p^2 = .04$ [0.00; 0.17], phase, $F(1, 46) = 1.46$, $p = .234$, $\eta_p^2 = .03$ [0.00; 0.15], or their interaction, $F(1, 46) = 0.39$, $p = .537$, $\eta_p^2 < .01$ [0.00; 0.10], suggesting that there were no meaningful differences between the anxiety and the neutral context (see Fig. 3.3).

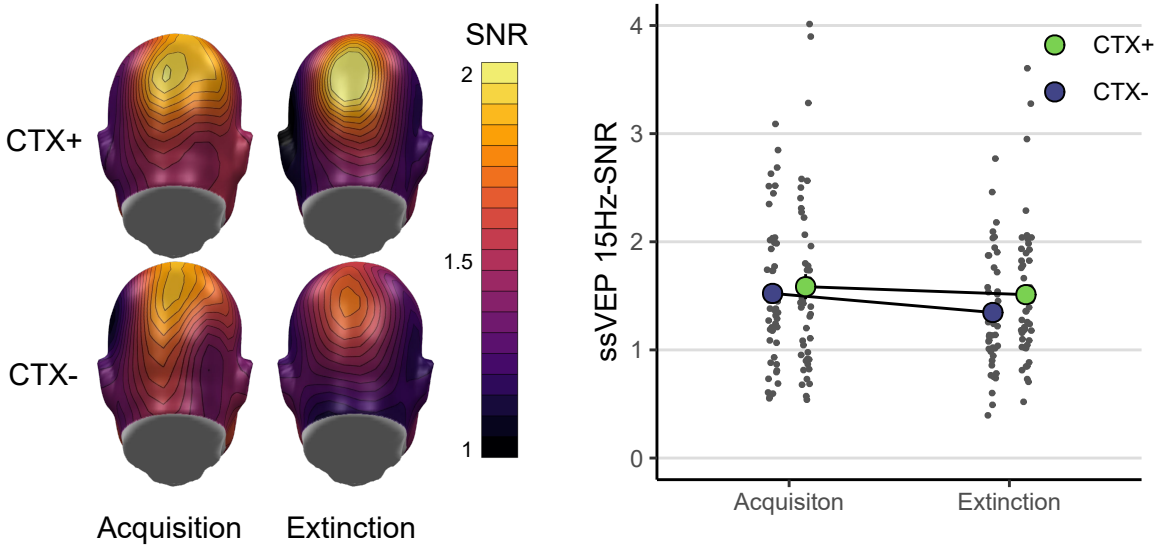

Figure 3.3: Mean (±SEM) visuocortical responses (right) and their topographies (left) to the context onsets during acquisition and extinction.

Central cue responses

During acquisition, visuocortical engagement to the central cues was neither modulated by cue condition, $F(1, 44) = 0.01$, $p = .935$, $\eta_p^2 < .01$ [0.00; 0.01], context condition, $F(1, 44) = 2.09$, $p = .155$, $\eta_p^2 = .05$ [0.00; 0.17], or their interaction,

$F(1, 44) = 0.21$, $p = .650$, $\eta_p^2 < .01$ [0.00; 0.08]. Similarly, no effects were found during extinction for cue condition, $F(1, 44) = 0.96$, $p = .333$, $\eta_p^2 = .02$ [0.00; 0.13], context condition, $F(1, 44) = 0.00$, $p = .996$, $\eta_p^2 < .01$ [0.00; 0.00], or their interaction, $F(1, 44) = 1.14$, $p = .292$, $\eta_p^2 = .03$ [0.00; 0.14] (see Fig. 3.4 and Fig. 3.5).

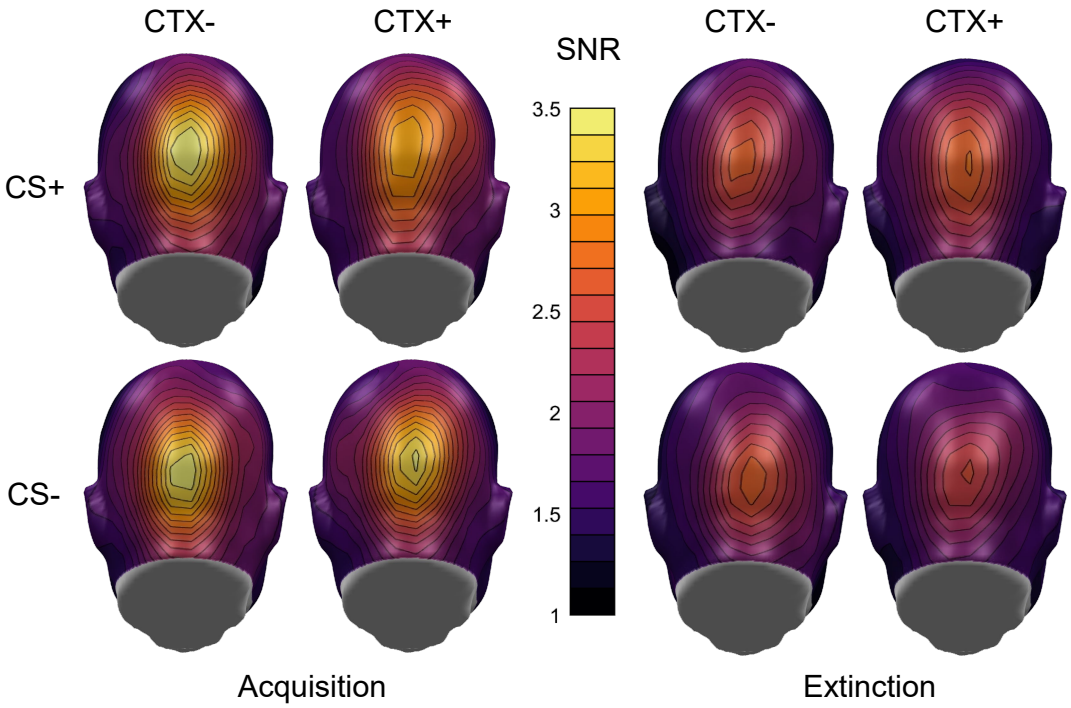

Figure 3.4: Topographies of the mean ssVEP amplitudes to the central cues during the acquisition phase (left) and the extinction phase (right).

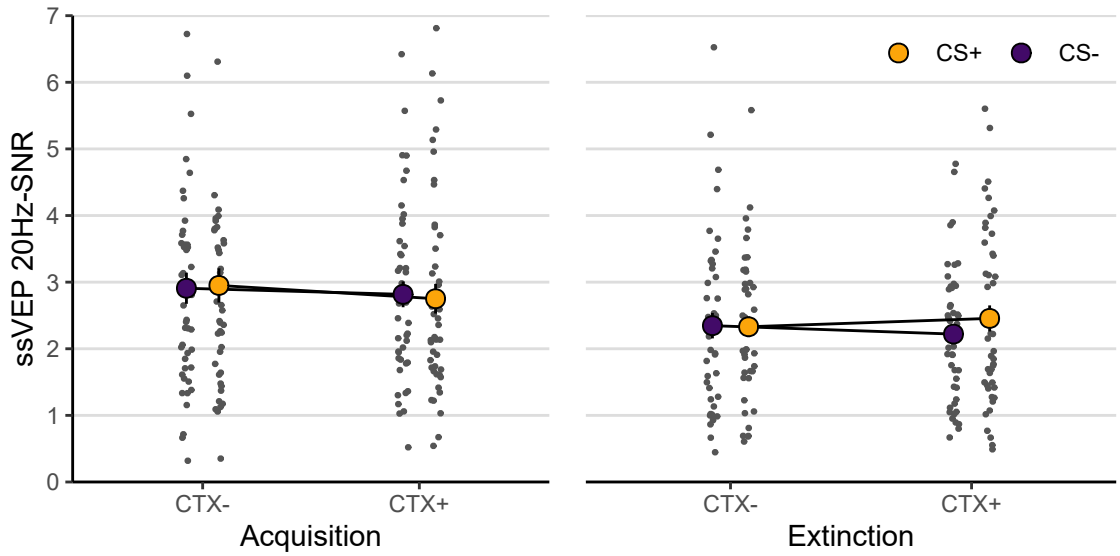

Figure 3.5: Visuocortical responses (±SEM) to the central cues during acquisition (left) and extinction (right).

Cue-locked context responses

The ANOVA for the central-cue-locked visuocortical engagement to the contexts revealed a marginal significant main effect of context, $F(1, 44) = 3.36$, $p = .074$, $\eta_p^2 = .07$ [0.00; 0.21], without a main effect of cue, $F(1, 44) = 1.81$, $p = .185$, $\eta_p^2 = .04$ [0.00; 0.16], or a Cue x Context interaction, $F(1, 44) = 1.99$, $p = .165$, $\eta_p^2 = .04$ [0.00; 0.17], indicating higher SNRs for the anxiety context compared to the neutral context - independent of which central-cue condition was presented (see Fig. 3.6 and Fig. 3.7).

During extinction, the effects of context, $F(1, 44) = 0.14$, $p = .706$, $\eta_p^2 < .01$ [0.00; 0.07], and cue were not significant, $F(1, 44) = 0.00$, $p = .971$, $\eta_p^2 < .01$ [0.00; 0.00], however, the ANOVA yielded a marginally significant interaction of cue and context, $F(1, 44) = 3.62$, $p = .064$, $\eta_p^2 = .08$ [0.00; 0.22]. Visual inspection of the interaction revealed that the anxiety context elicited slightly higher SNRs, when the CS- compared to the CS+ was presented, while the neutral context received amplified visuocortical processing during CS+ compared to CS- presentations. However, post-

hoc t-tests showed no significant differences between single levels of the interaction, $ps > .16$. Interestingly, exploratory t-tests indicated that cue-locked visuocortical responses to the anxiety context, $t(44) = 2.06$, $p = .046$, $d = 0.31$ $[< 0.01; 0.60]$, but not to the neutral context, $t(44) = 0.26$, $p = .797$, $d = 0.04$ $[-0.25; 0.33]$, diminished from acquisition to extinction.

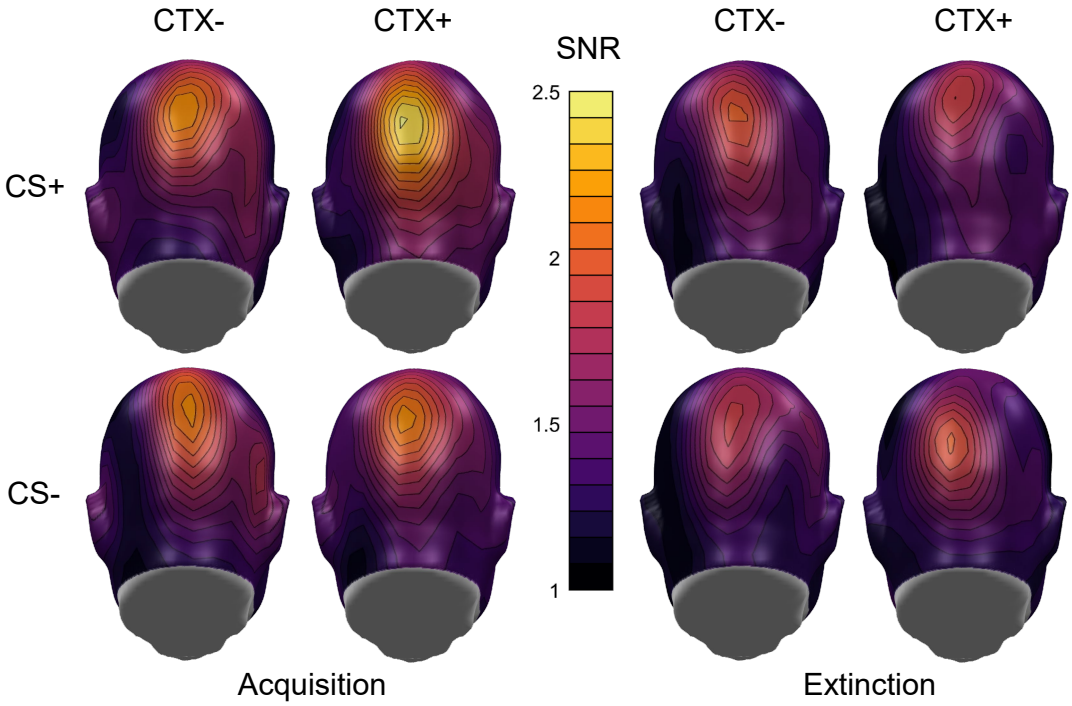

Figure 3.6: Topographies of the mean ssVEP amplitudes to the contexts during the central-cue presentation in the acquisition phase (left) and the extinction phase (right).

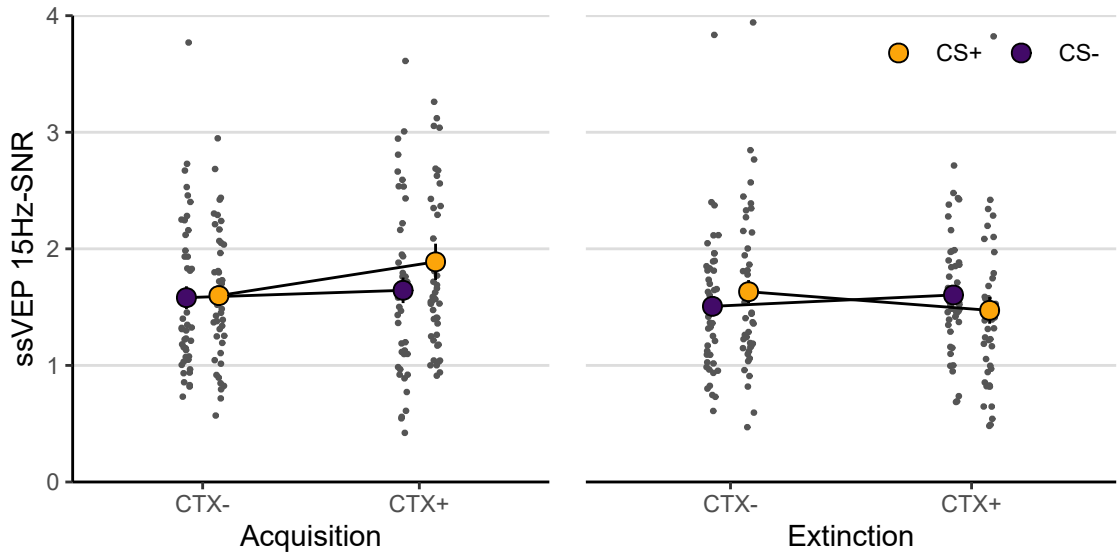

Figure 3.7: Visuocortical responses (±SEM) to the contexts during the central-cue presentation in acquisition (left) and extinction (right).

3.3.2 Skin conductance responses

Context responses

The ANOVA of the mean skin conductance responses to the context onsets revealed a significant main effect of context condition, $F(1, 44) = 26.68$, $p < .001$, $\eta_p^2 = .38$ $[0.19; 0.52]$, the CTX+ elicited stronger SCRs than the CTX-. Neither the main effect of phase, $F(1, 44) = 0.02$, $p = .885$, $\eta_p^2 < .01$ $[0.00; 0.04]$, or their interaction, $F(1, 44) = 0.10$, $p = .750$, $\eta_p^2 < .01$ $[0.00; 0.07]$, were significant, suggesting stable differences between the conditions during acquisition and extinction (see Fig. 3.8).

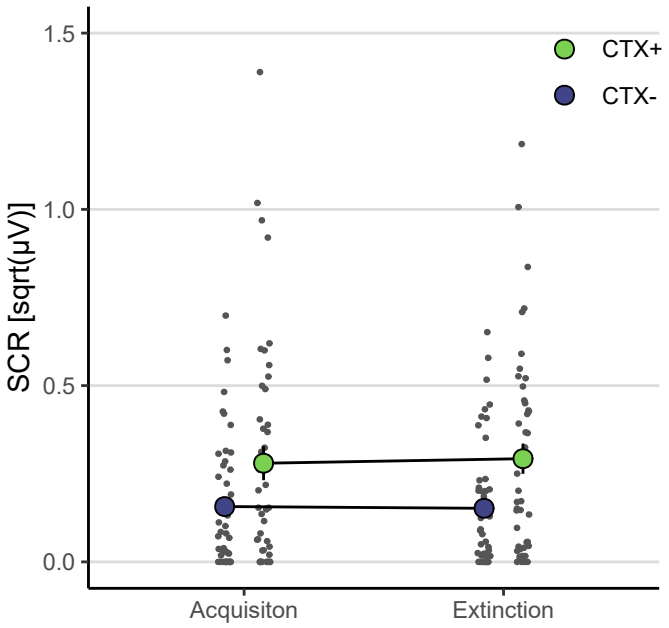

Figure 3.8: Mean skin conductance responses (±SEM) to the context onsets during acquisition and extinction.

Central cue responses

The ANOVA for skin conductance responses to the central cues during acquisition revealed a significant main effect of cue condition, $F(1, 44) = 9.22$, $p = .004$, $\eta_p^2 = .17$ $[0.04; 0.33]$, while there was no effect of context condition, $F(1, 44) = 0.00$, $p = .975$, $\eta_p^2 < .01$ $[0.00; 0.00]$, or a cue x context interaction, $F(1, 44) = 0.87$, $p = .356$, $\eta_p^2 = .02$ $[0.00; 0.13]$, indicating higher SCRs to the CS+ compared to the CS-, independent of the context condition.

During extinction the effect of cue condition remained significant, $F(1, 44) = 17.74$, $p < .001$, $\eta_p^2 = .29$ $[0.11; 0.44]$, the CS+ elicited stronger SCRs than the CS-. In addition, a main effect of context condition was found, $F(1, 44) = 5.37$, $p = .025$, $\eta_p^2 = .11$ $[0.01; 0.26]$. The interaction of cue and context condition was not significant, $F(1, 44) = 0.68$, $p = .413$, $\eta_p^2 = .02$ $[0.00; 0.12]$. This effect suggests stronger SCRs responses to both central cues, when they were presented in the CTX+ compared to

the CTX- (see Fig. 3.9).

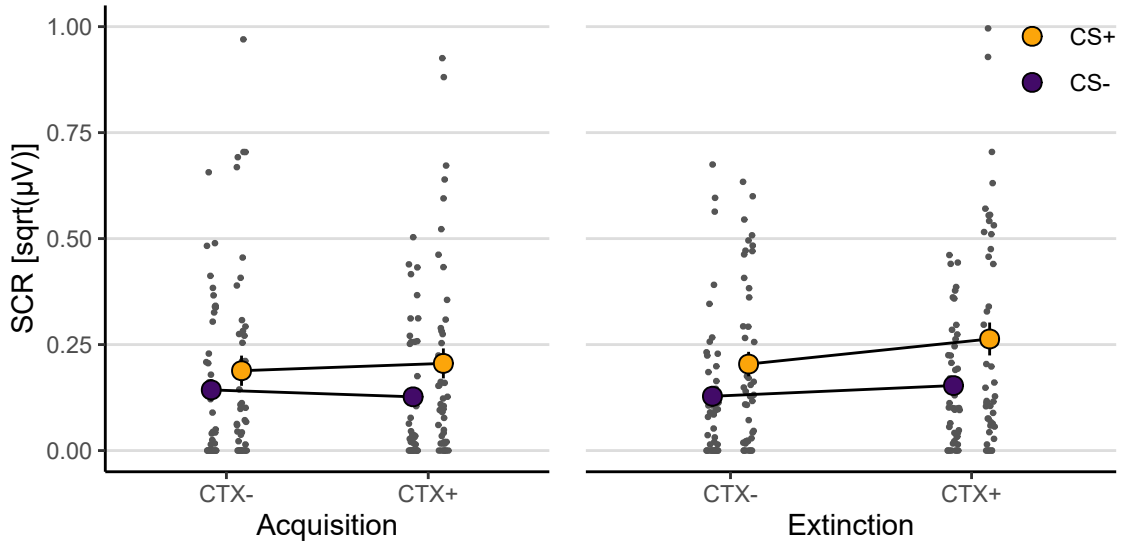

Figure 3.9: Mean skin conductance responses (±SEM) to the central cues during acquisition (left) and extinction (right).

3.3.3 Aversive Ratings

Context ratings

The ANOVA for threat and US-expectancy ratings of the contexts (see Fig. 3.10) revealed significant main effects of context, threat: $F(1, 44) = 73.23$, $p < .001$, $\eta_p^2 = .62$ $[0.46; 0.72]$; US-expectancy: $F(1, 44) = 179.26$, $p < .001$, $\eta_p^2 = .80$ $[0.70; 0.85]$, and phase, threat: $F(1, 44) = 51.39$, $p < .001$, $\eta_p^2 = .54$ $[0.36; 0.65]$; US-expectancy: $F(1, 44) = 131.70$, $p < .001$, $\eta_p^2 = .75$ $[0.63; 0.81]$, which were further qualified by a significant interaction of context and phase, threat: $F(1, 44) = 49.50$, $p < .001$, $\eta_p^2 = .53$ $[0.35; 0.64]$; US-expectancy: $F(1, 44) = 169.90$, $p < .001$, $\eta_p^2 = .79$ $[0.69; 0.84]$. During acquisition, threat and US-expectancy ratings were higher for the CTX+ compared to the CTX-, threat: $t(44) = -8.46$, $p < .001$, $d = -1.26$ $[-1.65; -0.86]$; US-expectancy: $t(44) = -15.04$, $p < .001$, $d = -2.24$ $[-2.79; -1.69]$. These differences diminished during extinction but stayed significant, threat: $t(44) = -5.21$, $p < .001$, $d = -0.78$

$[-1.11; -0.44]$; US-expectancy: $t(44) = -4.67$, $p < .001$, $d = -0.70$ $[-1.02; -0.37]$.

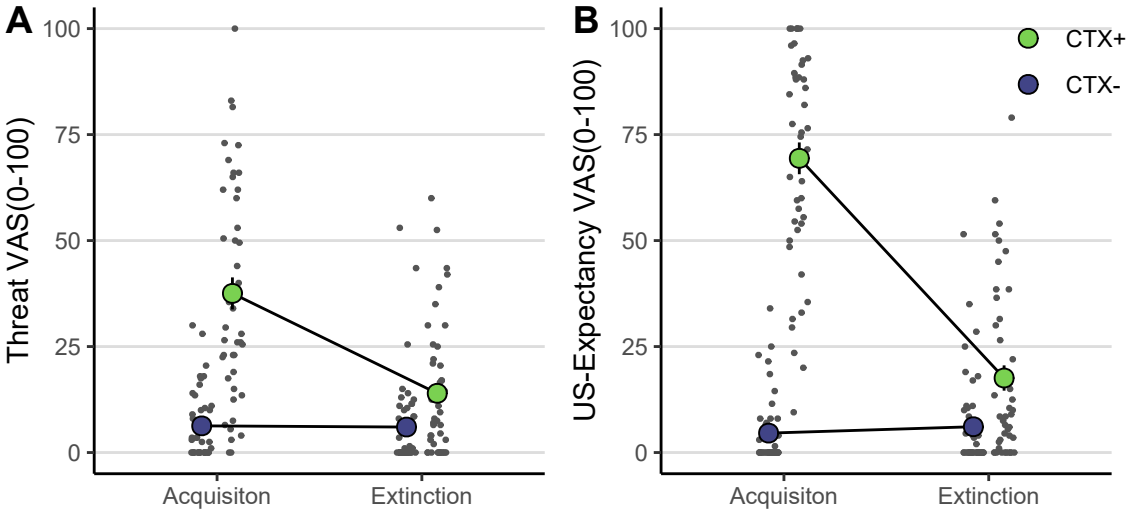

Figure 3.10: (A) Threat and (B) US-expectancy ratings (±SEM) of the contexts during acquisition and extinction.

Central cue ratings

The ANOVA for the threat ratings of the central cues during acquisition yielded a significant main effect of cue, $F(1, 44) = 60.51$, $p < .001$, $\eta_p^2 = .58$ $[0.41; 0.68]$, and context condition, $F(1, 44) = 57.29$, $p < .001$, $\eta_p^2 = .57$ $[0.39; 0.67]$. The CS+ was rated as more threatening as the CS-. Both central cues were perceived as more threatening when they were presented in the anxiety context compared to the neutral context (see Fig. 3.11), however there was no significant interaction, $F(1, 44) = 1.09$, $p = .302$, $\eta_p^2 = .02$ $[0.00; 0.14]$. During extinction, the main effect of cue,$F(1, 44) = 24.84$, $p < .001$, $\eta_p^2 = .36$ $[0.17; 0.50]$, and context condition, $F(1, 44) = 24.28$, $p < .001$, $\eta_p^2 = .36$ $[0.17; 0.50]$ diminished but remained significant. Again, there was no Cue x Context interaction, $F(1, 44) = 0.93$, $p = .341$, $\eta_p^2 = .02$ $[0.00; 0.13]$.

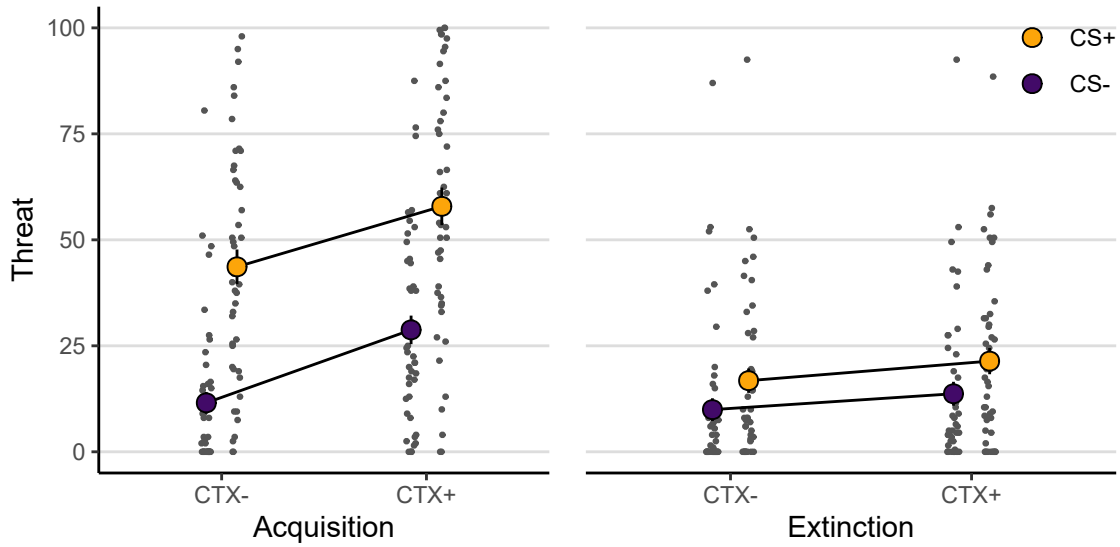

Figure 3.11: Threat ratings (±SEM) of the central cues during acquisition (left) and extinction (right).

Regarding US-expectancy ratings, there was a main effect of cue, $F(1, 44) = 215.68$, $p < .001$, $\eta_p^2 = .83$ [0.74; 0.87], indicating higher US-expectancy ratings for the CS+ than for the CS-. In addition, the ANOVA yielded a main effect of context condition, $F(1, 44) = 38.10$, $p < .001$, $\eta_p^2 = .46$ [0.27; 0.59], which was further qualified by a Cue x Context interaction, $F(1, 44) = 12.78$, $p < .001$, $\eta_p^2 = .23$ [0.07; 0.38]. The US-expectancy was generally higher for central cues during the anxiety context compared to the neutral context. However, the difference between CS+ and CS- was larger during the CTX- than during the CTX+, which is most likely due to a ceiling effect for the CS+ during the CTX+ (see Fig. 3.12). This notion gets further support from the results of the extinction phase, as the interaction could not be retrieved, $F(1, 44) = 3.62$, $p = .064$, $\eta_p^2 = .08$ [0.00; 0.22], while the main effect of cue, $F(1, 44) = 31.34$, $p < .001$, $\eta_p^2 = .42$ [0.23; 0.55], and context, $F(1, 44) = 15.26$, $p < .001$, $\eta_p^2 = .26$ [0.09; 0.41], remained significant.

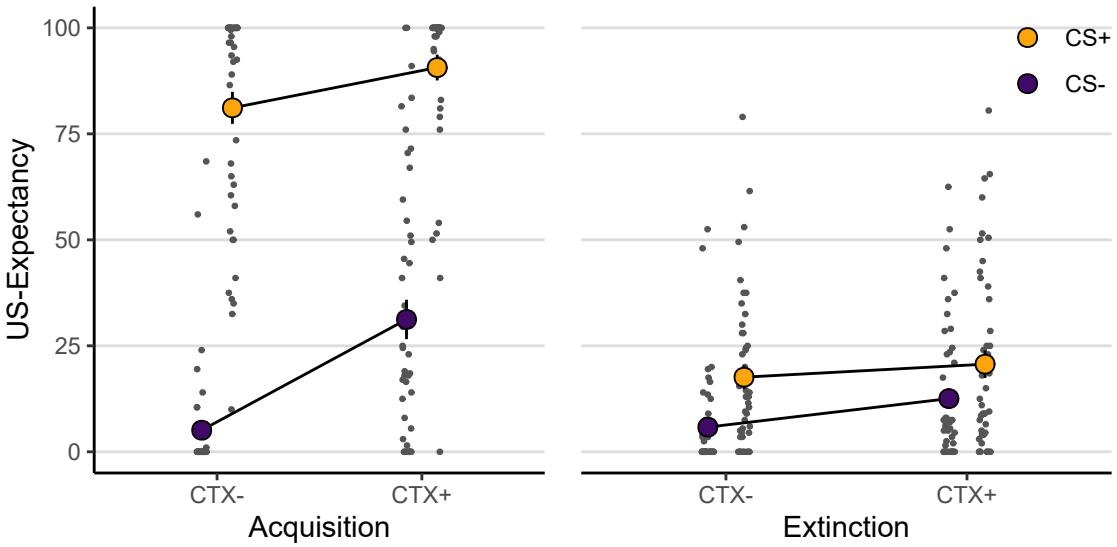

Figure 3.12: US-expectancy ratings (±SEM) of the central cues during acquisition (left) and extinction (right).

3.4 Discussion

The main goal of the present study was to test a novel paradigm, which orthogonally combines cue and context conditioning to investigate the interaction of fear and anxiety. To obtain convergent measures from different threat processing systems, steady-state visual evoked potentials (ssVEPs) were quantified as an index of visuocortical activity, while skin conductance responses and behavioral ratings were collected as measures of psychophysiological and subjective defensive responding.

Regarding measures of defensive responding, results demonstrated successful context conditioning. The anxiety context was rated as more threatening and more associated with an US than the neutral context. In addition, the onset of the anxiety context elicited enhanced SCRs compared to the onset of the neutral context. These results are in line with previous context conditioning studies (e.g. Andreatta et al., 2015; Glotzbach-Schoon et al., 2015; Glotzbach-Schoon, Tadda, et al., 2013), suggesting that potential threat induces anxiety and facilitates defensive mechanisms

in order to prepare for upcoming danger (Lang et al., 2000).

Similarly, the central fear cue elicited higher SCRs and obtained higher ratings of perceived threat and US-expectancy than the neutral cue. Importantly, these effects were found independent of the context condition, indicating successful fear conditioning in general. Thus, these findings contribute to a vast body of research, demonstrating elevated defensive responses to actual threat (Ojala & Bach, 2020).

Crucially, behavioral ratings to the central cues were elevated when they were presented during the anxiety compared to the neutral context. Furthermore, transient SCRs to the central cues were higher during the anxiety than during the neutral context as well. It is important to mention, however, that there was no significant interaction between cue and context conditioning. Consequently, anxiety-induced potentiation was not specific to fear cues, but could be observed for both central cues. Moreover, the effect of context conditioning was additive to the effect of fear conditioning. These results have important implications for the interaction of fear and anxiety. First, fear and anxiety do not seem to be mutually exclusive. (Fanselow, 2018; Hamm, 2020; Lang et al., 2000; Mobbs et al., 2015). If either anxiety or fear could be active at a given time, the magnitude of the transient responses to the fear cue would have been the same when presented during the anxiety as compared to during the neutral context. Second, fear responses during the anxiety context were stronger than during the neutral context, however, the effect of anxiety was not selective to fear, but generalized to both central cues, implying a general additive mechanism between fear and anxiety. Critically, even though the magnitude of the behavioral ratings generally diminished, the additive effects of fear and anxiety remained stable throughout extinction learning.

An additive model of fear and anxiety fits well with the main idea of the threat-imminence model. During the pre-encounter stage, potential danger elicits anxiety, which mobilizes some -but not all- defensive resources to prepare for an upcoming

threat. Entering the post-encounter stage, additive effects of fear provide further resources, as now an actual attack is more imminent. Thus, additive interactions between fear and anxiety might represent a higher-level mechanism that efficiently organizes defensive resources according to the characteristics of the current environment.

Furthermore, this model emphasizes the functional relevance of anxiety and fear in facilitating defensive responses. From an evolutionary perspective, potentiated defensive responses increase the organism's chances of survival (Blanchard & Blanchard, 1989; Mobbs et al., 2015). It is unclear, however, why defensive responding would be organized in this additive, multi-staged manner. As failure results in certain death, wouldn't it be best for the organism to deploy as many resources as possible regardless of already expecting the danger? Addressing this question, two hypotheses seem plausible: On the one hand, since potential threat situations are more frequent than actual threat, always responding in an 'all-or-nothing' fashion would be very cost-intensive, resulting in a depletion of vital metabolic resources (LeDoux, 2012). Consequently, hierarchically responding to threat increases the efficiency of the organism's survival behavior (Mobbs et al., 2015). On the other hand, it is quite possible that fear and anxiety do not tap into the same pool of resources. Fear is a rapid and phasic response to threat (Davis et al., 2010). Because of that, however, it might only be able to mobilize the fraction of resources that are quickly accessible. In contrast, anxiety is a more sustained response, enabling the organism to draw on additional, stored resources. This view is also in line with recent neurophysiological models, suggesting short-lived central amygdala outputs during acute threat and more slowly recruited BNST outputs during potential threat (Perusini & Fanselow, 2015). More research is needed, however, to elucidate the interplay of these pathways, especially on the neural and behavioral output level.

In the present study, it also important to mention that the effect of context con-

ditioning on SCRs to the central cues could only be observed during extinction, while there was only a descriptive effect during acquisition. The absence of this effect during acquisition is probably due to methodological reasons: The onset of contexts elicits SCRs as well. In this case, the onset of the anxiety context also prompted higher SCR amplitudes than the neutral context. It is well known, however, that the tails of skin conductance responses are relatively long, lasting about 30 seconds (Bach, Flandin, Friston, & Dolan, 2010). Critically, consecutive skin conductance responses during this interval are attenuated. Moreover, SCRs to the unpredictable US during the anxiety context may have further confounded the analysis of the transient cue responses, especially when the USs were presented prior to the central cue onsets. Even though there are modeling approaches trying to reduce the impact of overlapping responses (Bach et al., 2010), future studies need to pay attention to the timing of the stimulus presentations, especially for cue in context conditioning paradigms, and should consider the use of more phasic measures of psychophysiological arousal, like pupil size responses (Ojala & Bach, 2020).

Regarding visuocortical activity, the present study revealed no consistent effects. It is not surprising, however, to find divergent outcomes between measures of threat detection mechanisms and measures of defensive responding, especially given their specific functions within the threat processing cascade. Similar inconsistencies have been reported and discussed before (e.g. McTeague et al., 2015; Ahrens et al., 2015). Results of the present study revealed marginally significantly higher visuocortical responses to the anxiety in comparison to the neutral context during presentations of central cues in general. This finding would be in line with the notion of heightened visuocortical processing as an index of hypervigilance during sustained contextual anxiety. Furthermore, exploratory analysis of cue-locked visuocortical responses from acquisition to extinction demonstrated a reduction of ssVEP amplitudes to the anxiety context, indicating a visuocortical correlate of extinction learning. However,

these effects were rather small and could not be obtained during the onset of the contexts. Nor could the present study find any effects of cue conditioning on ssVEP amplitudes and thus revealed no further evidence for the visuocortical correlate of selective attention.

There are two potential reasons for the absence of detectable threat-related changes in visuocortical responding. First, the same type of US (loud, unpleasant noises) has been used for cue and context conditioning. Accordingly, participants were confronted with many US presentations and in a highly complex pattern. Even though participants were instructed about the exact US-contingencies, the complexity of the paradigm could have led to interferences regarding the aversive learning. A very similar pattern could be observed in a recent study, which investigated the influence of startle-probes during fear conditioning (Sjouwerman, Niehaus, Kuhn, & Lonsdorf, 2016). The authors demonstrated reduced fear learning in a group with versus a group without startle-probes. Importantly, startle-probes are usually perceived as aversive and are administered at several time points, paralleling aspects of potential threat. Yet, in the present study, ratings and skin conductance responses showed successful differential conditioning. There is plenty of evidence, however, that these measures are sensitive to verbal instructions (Sevenster, Beckers, & Kindt, 2014; Weike et al., 2005; Weike, Schupp, & Hamm, 2007). At the same time, it could be demonstrated that ssVEP amplitudes are primarily driven by experience (Moratti & Keil, 2009; Moratti et al., 2006) and that they are even independent of conscious expectations (Yuan et al., 2018). Consequently, ssVEP amplitudes might be particularly sensitive to interferences by two concurrent aversive learning procedures, i.e. cue and context conditioning.

Second, analysis of the electrocortical signal revealed rather low ssVEP signal-to-noise ratios, with mean SNRs ranging from 1.5 for context responses to 3 for cue responses (see Fig. 3.2). Please note that SNRs of 1 imply the absence of a detectable

ssVEP signal. Crucially, ssVEP with low SNRs might also be less sensitive to psychological modulations. In the present study, there are several methodological factors that could have impacted the ssVEP signal. The frequency tagging technique enables the disentangling of visuocortical responses to two concurrently presented visual stimuli. However, competition for attentional resources between these stimuli results in a reduced ssVEP signal as compared to an individual presentation (e.g. Wieser & Keil, 2014; Boylan et al., 2019; Kastner et al., 2015), which is especially relevant if both stimuli gained motivational significance through aversive learning. Yet, the present study is unable to quantify the competition as there was no phase, in which the central cues were presented individually. Another important factor is that the SNR increases with a growing number of experimental trials, which has been reported for ERPs (Thigpen et al., 2017) and also extends to ssVEPs, albeit to a lesser degree, as a special variant of an ERP. In the present study, a maximum of 12 trials of central cue presentation per context could have been obtained, which is further reduced by a mean rejection rate of 25% due to artifacts. Thus, future studies need to increase the number of trials to obtain satisfactory SNRs for combined cue and context conditioning paradigms.

In conclusion, the present study demonstrated first evidence for additive effects of fear and anxiety in defensive response to actual and potential threat. No effects were found for visuocortical responding, which might be explained by low trial numbers in combination with a complex aversive cue and context conditioning design, resulting in generally low electrocortical signal-to-noise ratios. Therefore, the aim of Study 3 is to replicate the findings of additive effects for measures of threat responding, while adjusting methodological factors to facilitate the investigation of visuocortical activity. To this end, Study 3 utilizes an orthogonal combination of cue conditioning and a threat-of-shock paradigm as an alternative method to induce potential threat.

Study 3: The effect of anticipatory anxiety on visuocortical engagement to conditioned threat stimuli

4.1 Introduction

Fear is a phasic response to acute threat, while anxiety is a sustained state of apprehension during potential threat (Davis et al., 2010; Sylvers et al., 2011). And even though they have been regarded as two separate emotional states, the previous study demonstrated first evidence for their interaction, i.e. additive effects between fear and anxiety. The additive model of fear and anxiety is well in line with the threat-imminence model (Blanchard & Blanchard, 1989; Fanselow & Lester, 1988; Lang et al., 2000) and might represent an economic and efficient mechanism to organize defensive behavior. Equally important are the different functions of attention during fear and anxiety. It has been suggested that fear prompts selective attention, whereas anxiety is associated with heightened vigilance (Lang et al., 2000). However, there is still little evidence for these attentional mechanisms on a direct, neurophysiological level and while the previous study revealed additive effects for measures of defensive responding, it failed to find changes of visuocortical activity during acute or potential threat.

The absence of these effects could be due to methodological reasons, which have been discussed previously. In brief, the simultaneous cue and context conditioning

could have led to interferences between learning mechanisms. To reduce complexity, participants were instructed about the cue and context US-contingencies, but a potential impact of combined versus single conditioning could not be quantified. Thus, to reduce interference effects on learning processes and to estimate the impact of concurrent context and central cue versus individual presentation, Study 3 implemented a separate cue acquisition phase prior to the orthogonal cue and context conditioning test phase. In addition, Study 3 generally increased the number of trials to ensure high signal-to-noise ratios and reliabilities for visuocortical as well as psychophysiological measures (Boucsein et al., 2012; Thigpen et al., 2017). Finally, in the previous study, unpredictable US were sometimes administered directly prior to a central cue onset, leading to systematic artifacts on SCR and visuocortical responding to central cues in the anxiety context.

To address this issue, Study 3 utilized a 'threat-of-shock' paradigm as an alternative means to induce potential threat (e.g. Bublatzky, Flaisch, Stockburger, Schmalzle, & Schupp, 2010; Bublatzky, Guerra, Pastor, Schupp, & Vila, 2013). In a threat-of-shock paradigm, participants are usually instructed to expect a small number of highly aversive stimuli during one context (anticipatory anxiety context), while there will be not aversive events during a second context (neutral context). Throughout the experiment, however, participants actually never experience any aversive stimuli. Similar to context conditioning, it could be demonstrated that verbally instructed anxiety contexts are associated with enhanced defensive responding on a wide range of different measures, including startle reflexes, skin conductance responses, cardiovascular activity and verbal reports of perceived threat (Bradley, Moulder, & Lang, 2005; Bublatzky et al., 2014, 2013; Grillon, Ameli, Woods, Merikangas, & Davis, 1991). And even in the absence of aversive experiences, verbally induced anxiety is very persistent over time, as the expectation of finally being confronted with an aversive event seems to increase with longer periods without aversive stimuli (Bublatzky

et al., 2014). Crucially, the design of Study 2 can be well modified to include a threat-of-shock paradigm. In addition, using verbally instructed anxiety instead of context conditioning, reduces US-related artifacts in the anxiety context. Yet, it is important to mention, that cue conditioning needs to rely on a different type of US (e.g. aversive noises) than the verbal instruction (e.g. anticipatory threat of electrical stimuli).

The main goals of the present study are to 1) replicate findings of Study 2 regarding the interaction of fear and anxiety and to 2) demonstrate further evidence for the visuocortical correlates of selective attention and hypervigilance during acute and potential threat. To this end, this study orthogonally combines cue conditioning and a threat-of-shock paradigm. Similar to Study 2, I expected elevated transient responses to the fear compared to a neutral cue and elevated sustained responses to the anxiety compared to a neutral context. Regarding the interaction of cue and context conditioning, I expected potentiated responses to the fear cue during the anxiety compared to the neutral context.

4.2 Methods and material

All methods and analyses have been preregistered at the Open Science Framework (`https://osf.io/672yd`).

4.2.1 Sample

In total forty subjects participated in the experiment (26 females, mean age ± SD: 24.1 ± 3.6 years). Participants were required to be between 18 and 35 years old. Exclusion criteria were any actual mental or neurological disorder and any family history of photic epilepsy. Participants completed the German versions of Spielberger's State-And-Trait Anxiety Inventory (STAI; Laux & Spielberger, 1981), the

Anxiety Sensitivity Index 3 (ASI-3; Reiss et al., 1986; Taylor et al., 2007) and the Intolerance of Uncertainty Scale (IUS; Buhr & Dugas, 2002). For a summary see Table 4.1. All participants gave written informed consent and were paid 12 € or received an equivalent in course credits. All procedures were approved by the ethics committee of the University of Würzburg.

Table 4.1: Descriptive statistics of the sample

	N	*M*	*SD*	*Mdn*	*Min*	*Max*
Age	40	24.10	3.60	24.0	19	32
STAI-S	40	34.40	6.44	34.0	24	51
STAI-T	40	36.23	8.87	34.5	22	68
ASI-3	40	16.27	8.83	15.5	2	47
IUS	40	44.58	10.81	42.0	29	71
US-Unpleasantness	40	6.38	1.61	6.5	3	10

Note: STAI = State-Trait-Anxiety-Inventory (S: Subscale State, T: Subscale Trait); ASI-3 = Anxiety Sensitivity Index 3; IUS = Intolerance of Uncertainty Scale.

4.2.2 Stimuli and Apparatus

The stimuli used for aversive cue conditioning were centrally presented, black-and-white Gabor patches, similar to Study 1. Two different orientations (-45° and 45°) indexed the CS+ and CS-, respectively. Gabor patches spanned a visual angle of 5.64° from a viewing distance of 100 cm. In order to evoke ssVEPs, Gabor patches were presented in flickering mode in 10 Hz. The context cues, indicating the anxiety and neutral context condition, consisted of two hexagons or dodecagons, presented peripherally ca. 1.88° of visual angle from the central Gabor patch, spanning visual angles of 3.75°. Contexts were presented in 7.5 Hz flickering mode to enable a

subsequent disentangling of the visuocortical responses evoked by central cues and contexts. All visual stimuli were counter-balanced for conditions across participants. The stimuli were presented on a black background on a 19-inch monitor (resolution = 1024 x 768 pixels) with a vertical refresh rate of 60 Hz, located ca. 100 cm in front of the participant. Presentation of all stimuli was controlled using Presentation Software (Neurobehavioral Systems, Inc., Albany, CA, USA).

For the cue conditioning, a 95 dB white noise bursts presented binaurally via headphones (Sennheiser, Wedemark, Germany) served as aversive unconditioned stimuli (US), similarly to Study 2. The US was presented once prior to the experiment and participants rated the US aversiveness on a scale ranging from 0 (not unpleasant) to 10 (very unpleasant), resulting in a mean (± SD) aversiveness of 6.38 ± 1.61. Again, there was no individual pain threshold calibration for the electro-tactile stimulus (Sperl et al., 2016). In addition, no electro-tactile stimuli were actually presented throughout the experiment.

4.2.3 Procedure

Participants were seated in a sound-attenuated, dimly lit testing room, where EDA-electrodes and the EEG-net were applied. The study consisted of two phases: fear acquisition and test (threat-of-shock) phase (see Fig. 4.1). During fear acquisition Gabor patches were presented for 5 s, separated by an ITI with a random duration of 8 - 10 s, consisting of a white fixation cross. Each condition was presented 30 times (60 cue presentations in total) in randomized order and US were delivered at the offset of the CS+ presentation with a reinforcement rate of 70% (= 21 US during fear acquisition). After fear acquisition subjective levels of threat and US-contingency were collected per cue condition via visual analogue scales, ranging from 0 = not threatening/ not likely to 100 = very threatening/ very likely.

Prior to the test phase, participants were briefed about the upcoming context

conditions. They were instructed that during the anxiety context (CTX+) at least one and up to three US could be presented, whereas no US would be presented during the neutral context (CTX-). During the test phase six blocks (3x CTX+ and 3x CTX-) were presented in pseudo-randomized order. Each block consisted of 20 central cue presentations (10x CS+ and 10x CS-; for 5 s) that were separated by an 8 - 10 s ITI. In sum, 30x CS+ and 30 CS- were presented during each context condition. US delivery was identical to the fear acquisition phase and was independent of the context condition. The contexts were presented throughout the full duration of a block and central cue presentation started 6 - 10 s after context onset. Consequently, each block lasted about 280 s. After every second block, subjective levels of threat, US-contingency and shock-expectancy was collected for each context condition and each combination of central cue and context condition.

4.2.4 Physiological data processing

Skin conductance was again recorded using two silver-silver chloride electrodes placed on the thenar and hypothenar eminences of the participants' left palmar surface. The signal was recorded with a V-Amp amplifier and Vision Recorder Software (Brain-Products Inc., Munich, Germany). A sampling rate of 1,000 Hz and a notch-filter at 50 Hz were applied. Analysis was then performed using Vision Analyzer Software (BrainProducts Inc., Munich, Germany). Trough-to-peak values within 1 s to 6 s after central cue onset were scored manually for each phase and condition (CS+ and CS- during fear acquisition and each combination of CS+ and CS- with CTX+ and CTX- during threat-of-shock phase). Individual responses smaller than 0.02 µS were scored as zero responses. All SCRs were square-root-transformed to account for eventual skewedness of the underlying data (Boucsein et al., 2012).

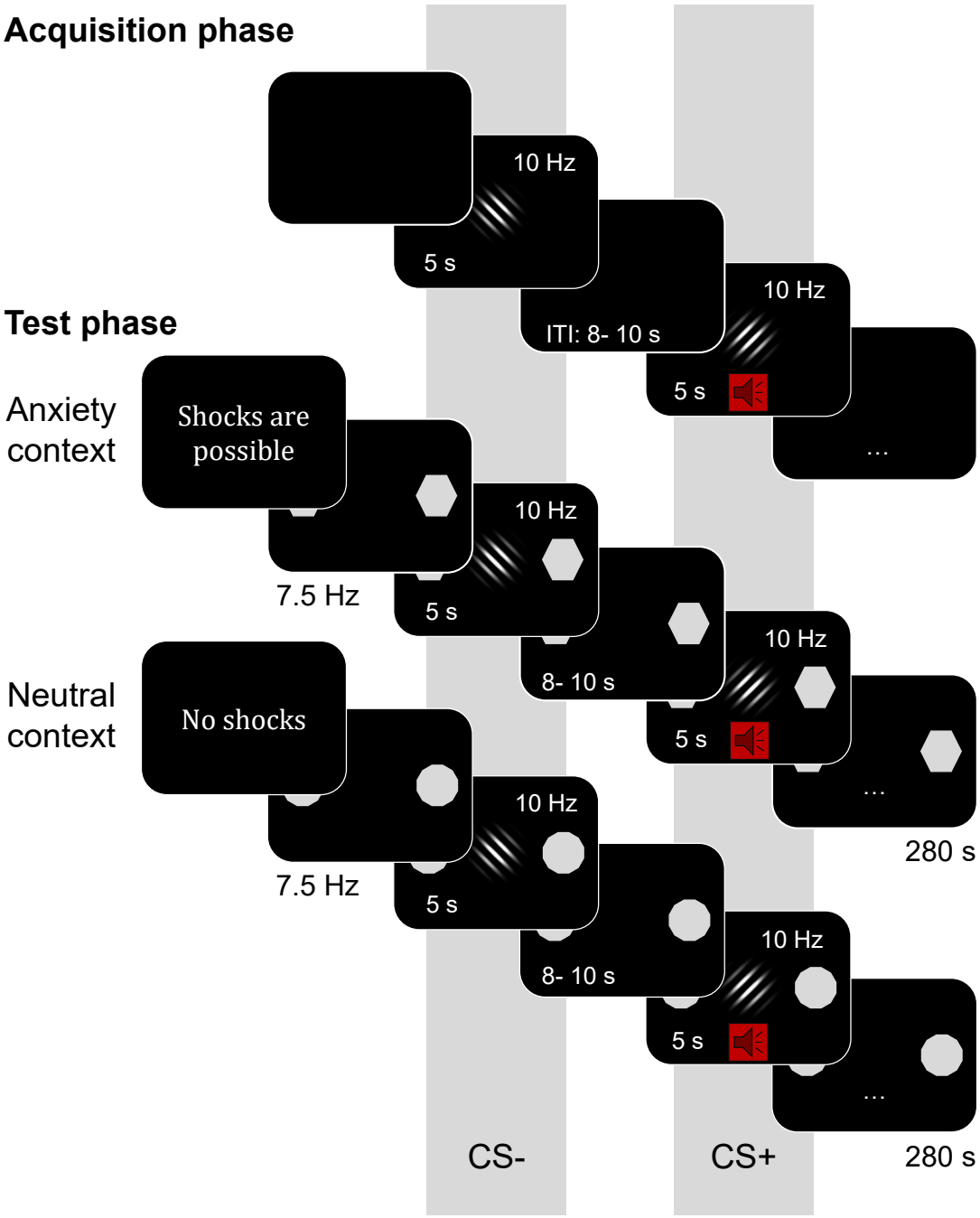

Figure 4.1: Experimental Design. During acquisition, two differently oriented (-45° and 45°) grating stimuli were presented for 5 s with an ITI of 8 to 10 s. At the offset of one of those stimuli (CS+) a loud noise blast was presented (US, reinforcement rate = 70%). During the test phase, the anxiety and neutral context were indicated by two different geometrical symbols, presented next to the grating stimuli. To disentangle visuocortical responses evoked by the contexts and central cues, contexts were presented with a flickering frequency of 7.5 Hz, while central cues were presented in 10 Hz.

4.2.5 EEG recording and data processing

To record electrocortical brain activity we used a 129 electrodes Electrical Geodesics System (EGI, Eugene, OR) referenced to the vertex electrode (Cz), with a sampling rate of 500 Hz and an online band-pass filter of 0.1-100 Hz. Electrode impedances were kept below 50 kΩ. Subsequent data processing occurred offline using the EMEGS software for Matlab. In a first step, all data were filtered using a 40-Hz low-pass filter (cut-off at 3 dB point; 45 dB/octave, 19th order Butterworth) and a 0.1-Hz high-pass filter (4th order Butterworth), before extracting epochs from 600 ms pre to 4,900 ms post cue-onset. Artifacts were identified and corrected according to the guidelines for the statistical correction of artifacts in dense array studies procedure (SCADS; Junghofer et al., 2000). The mean retention rate was 73.1 ± 17.1 % ($M \pm SD$).

Remaining epochs were averaged separately for the two cue conditions in the fear acquisition phase and the four combinations of cue and context conditions in the threat-of-shock phase. The current source densities (CSD) of the time-averaged data were calculated to account for volume conductance. The CSD approach employs a spatial Laplacian filter (the second spatial derivative) of the scalp potential to estimate the potential distribution at the cortical surface (Junghöfer et al., 1997). The CSD transformed data were then transformed into the frequency domain using a Fast-Fourier-algorithm on a time interval between 1000 and 4600 ms post cue-onset. The first 1000 ms after stimulus onset were omitted since effects of aversive conditioning on visuocortical responding are more prominent during a later stage of stimulus presentation due to early non-stationary components of the steady-state visual evoked potential (Miskovic & Keil, 2013b, 2013a).

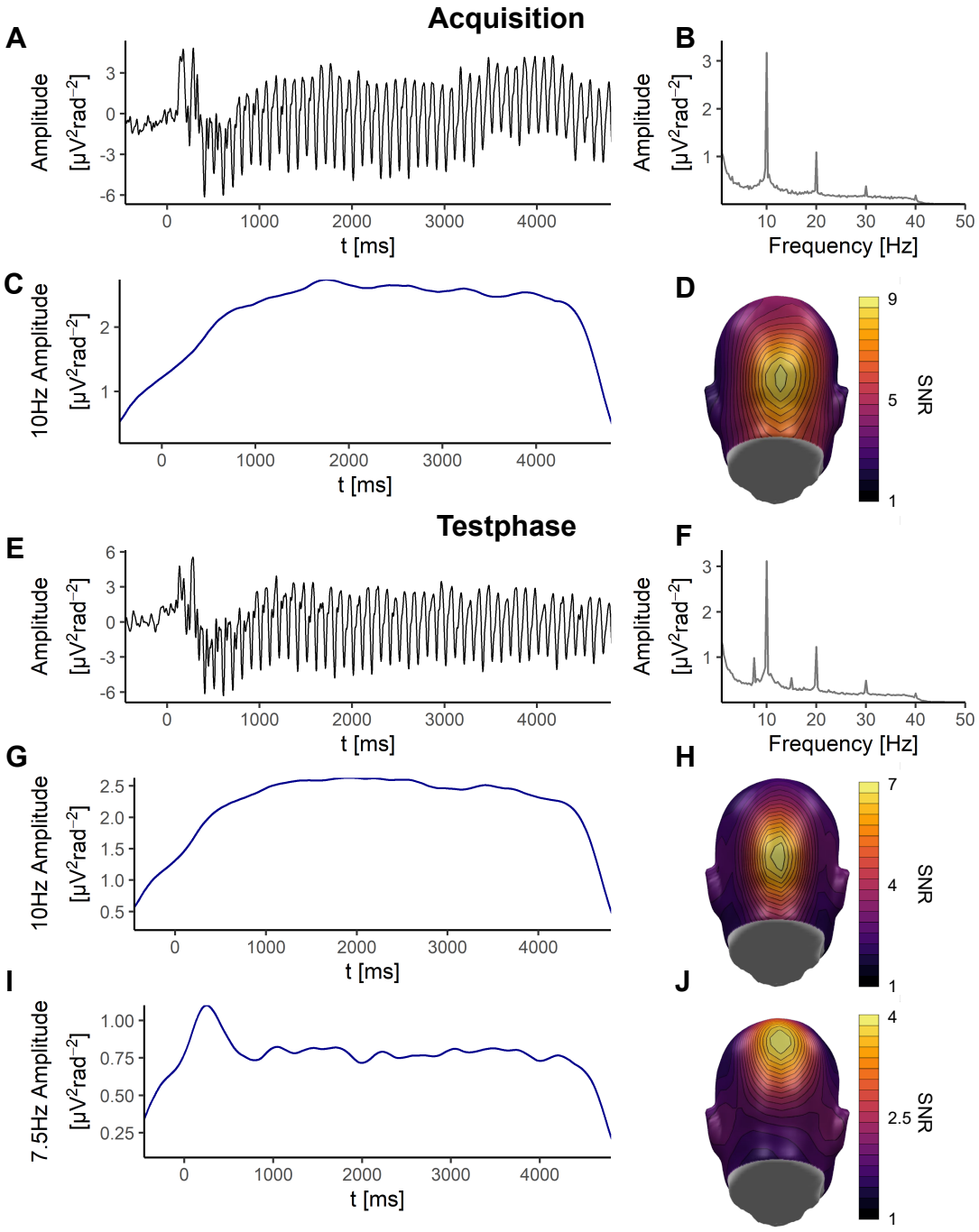

Figure 4.2: Characteristics of the grand averaged ssVEP signal during central cue presentations across all participants and conditions at Oz (sensor 75): Time-domain representation of the CSD-transformed ssVEP signal during acquisition (A) and test phase (E). Frequency-domain representation during acquisition (B) and test phase (F). Time-frequency analysis of the 10 Hz driving frequency during acquisition (C), test phase (G), and of the 7.5 Hz context frequency during test phase (I). Topographies of the 10 Hz SNR during acquisition (D), test phase (H) and of the 7.5 Hz context SNR during test phase (J).

The CSD-transformed ssVEP signals during acquisition and test phase for a representative electrode (Oz), the Fast-Fourier-Transformation on these ssVEPs, the time-frequency representations of the driving frequencies, and the topography of their SNRs averaged across all subjects and conditions are shown in Fig. 4.2. For statistical analysis, the ssVEP activity was pooled across the Oz and 7 surrounding electrodes (EGI sensors 70, 71, 72, 74, 75, 76, 82, 83; Wieser & Keil, 2014).

4.2.6 Statistical Analyses

Mean differences in ratings, SCRs and ssVEPs during fear acquisition were analyzed using two-sided t-tests, while analyses for the threat-of-shock phase were conducted via ANOVAs with the within-subject factors context (CTX+ vs CTX-) and cue condition (CS+ vs CS-). A significance level of 0.05 was used for all analyses and Greenhouse-Geisser correction was applied where appropriate. Throughout this manuscript, the uncorrected degrees of freedom, the corrected p values and the partial η^2 ($\eta_p{}^2$) or Cohen's d (d) and their 95% confidence interval are reported.

4.3 Results

4.3.1 Acquisition Phase

During acquisition, there was no difference between CS+ and CS- regarding ssVEP amplitudes, $t(39) = -1.12$, $p = .271$, $d = -0.18$ $[-0.49; 0.14]$ (but see Fig. 4.4). However, the CS+ elicited stronger SCRs than the CS-, $t(39) = -2.02$, $p = .050$, $d = -0.32$ $[-0.64; < 0.01]$, and after acquisition learning, the CS+ was rated as more threatening, $t(39) = -3.53$, $p = .001$, $d = -0.56$ $[-0.89; -0.22]$, and as more associated with the US than the CS-, $t(39) = -5.97$, $p < .001$, $d = -0.94$ $[-1.31; -0.57]$, (see Fig. 4.3).

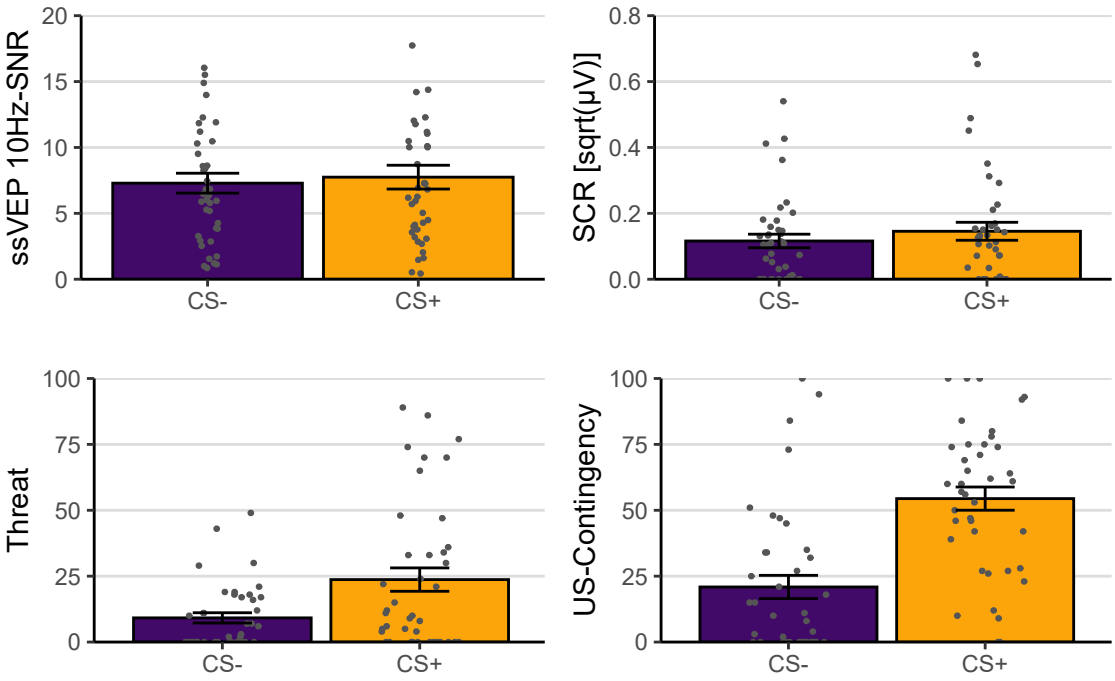

Figure 4.3: Mean threat responses (±SEM) during acquisition learning. There was no difference between CS+ and CS- for ssVEP amplitudes, but the CS+ elicited stronger SCRs and was associated with higher threat and US-contingency ratings than the CS-.

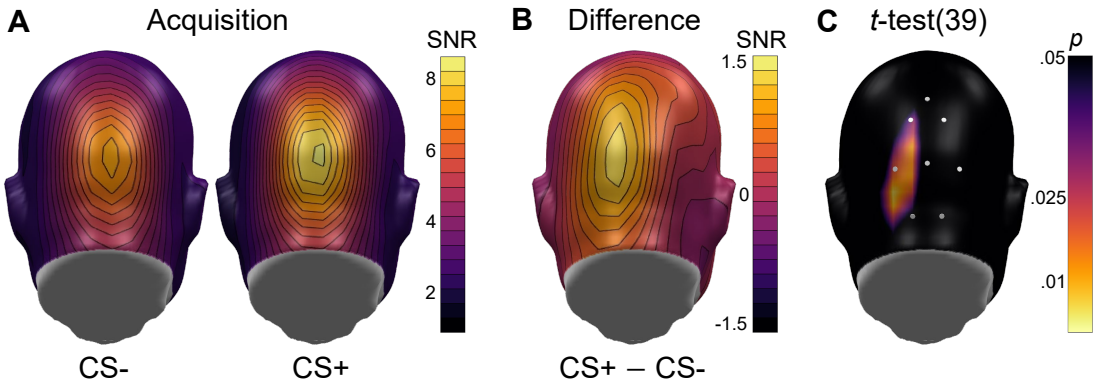

Figure 4.4: Topographies of the visuocortical responses to the central cues (A) and their difference (B) during acquisition. Figure C shows p-values resulting from paired t-tests (df = 39) per sensor. Only p-values smaller than 0.05 are depicted. Exploratorily, a significant cluster over the left occipital sensors (white sensors) could be identified, indicating increased visuocortical engagement to the CS+ compared to the CS-.

4.3.2 Test Phase

Steady-state visual evoked potentials

Central cue responses: During the test phase of the experiment, there was no significant effect of cue, $F(1, 39) = 0.38$, $p = .542$, $\eta_p^2 < .01$ $[0.00; 0.11]$, context, $F(1, 39) = 0.16$, $p = .695$, $\eta_p^2 < .01$ $[0.00; 0.08]$, nor any interaction, $F(1, 39) = 0.48$, $p = .490$, $\eta_p^2 = .01$ $[0.00; 0.12]$, for ssVEP amplitudes to the central cues (see Fig. 4.5).

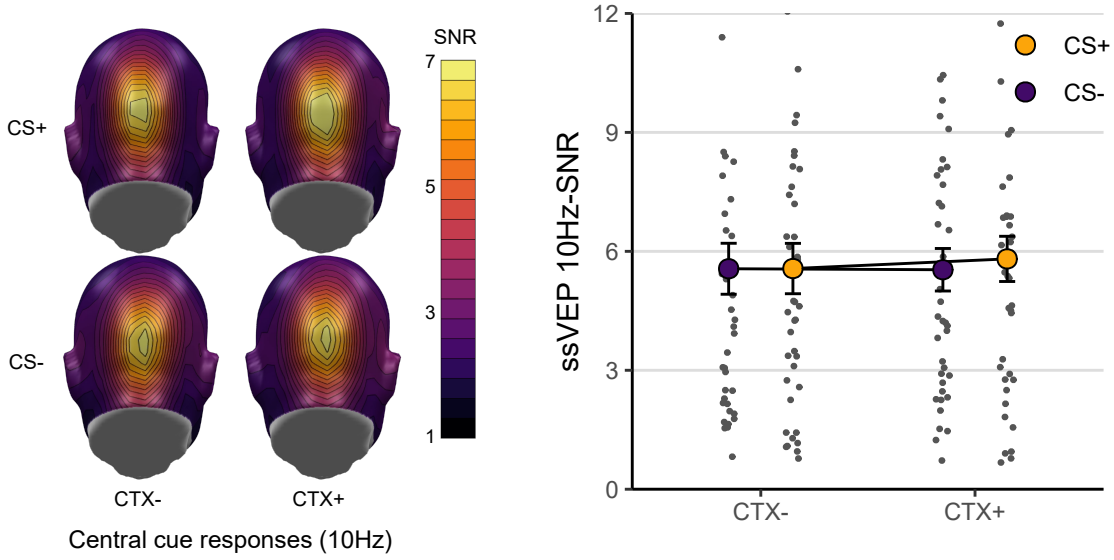

Figure 4.5: Topographies (left) and the corresponding mean (±SEM) visuocortical responses (right) to the central cues during the test phase.

Context responses: Analysis of the ssVEP amplitudes evoked by the contexts revealed a significant interaction of cue and context, $F(1, 39) = 7.23$, $p = .010$, $\eta_p^2 = .16$ $[0.02; 0.32]$. The main effects of cue, $F(1, 39) = 0.07$, $p = .788$, $\eta_p^2 < .01$ $[0.00; 0.07]$, and context, $F(1, 39) = 1.84$, $p = .182$, $\eta_p^2 = .05$ $[0.00; 0.18]$, were not significant. Post-hoc t-tests showed that when the CS+ was presented, the context in the neutral condition received facilitate sensory processing compared to the anxiety condition, $t(39) = 3.00$, $p = .005$, $d = 0.47$ $[0.14; 0.80]$ (see Fig. 4.6). No differential

processing of the contexts was found during the CS- presentation, $t(39) = -0.29$, $p = .772$, $d = -0.05$ $[-0.36; 0.26]$.

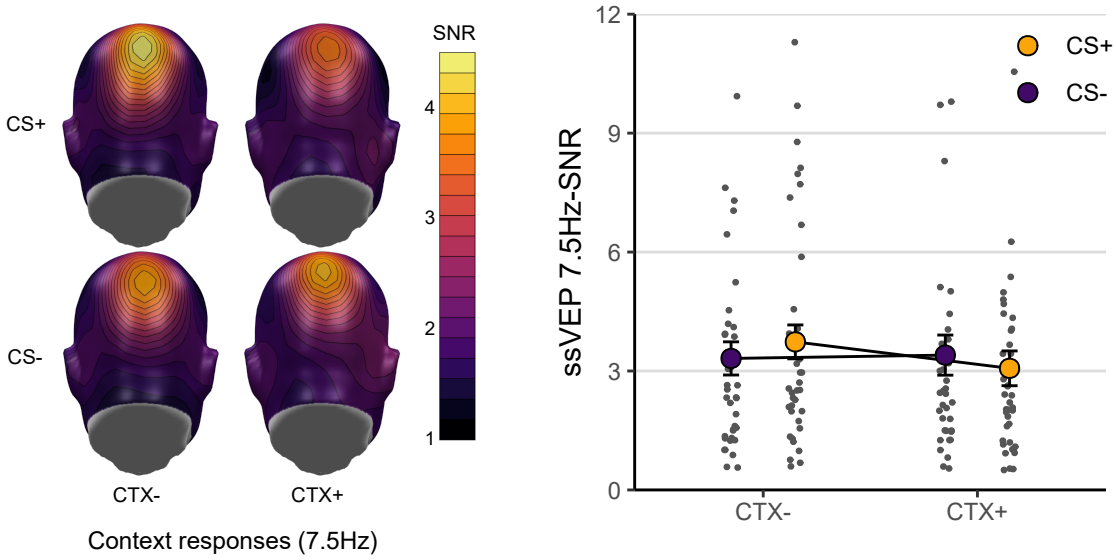

Figure 4.6: Topographies (left) and the corresponding mean (±SEM) visuocortical responses (right) to the contexts during the test phase.

Competition Index: The interaction effect for visuocortical responses to the contexts suggests a potential competition for attentional resources between central fear cues and contextual anxiety cues. To exploratory test this hypothesis, competition indices were calculated for each context condition via the following equations:

$$\begin{aligned} CI_{CTX+} = &(Cue\ ssVEP_{CS+|CTX+} - Cue\ ssVEP_{CS-|CTX+}) \\ &* (Context\ ssVEP_{CS+|CTX+} - Context\ ssVEP_{CS-|CTX+}) \end{aligned} \tag{4.1}$$

$$\begin{aligned} CI_{CTX-} = &(Cue\ ssVEP_{CS+|CTX-} - Cue\ ssVEP_{CS-|CTX-}) \\ &* (Context\ ssVEP_{CS+|CTX-} - Context\ ssVEP_{CS-|CTX-}) \end{aligned} \tag{4.2}$$

This index is negative if higher ssVEP responses to the CS+ compared to the CS- are accompanied by decreased amplitudes to the contexts during CS+ compared to CS- presentations (or vice versa. Crucially, stronger competition was expected during the anxiety compared to the neutral context as a function of motivational relevance.

However, paired t-tests for competition indices revealed no differences between the anxiety (mean ± SD: 0.097 ± 2.68) and neutral (mean ± SD: 0.03 ± 1.78) context, $t(39) = -0.13$, $p = .898$, $d = -0.02$ $[-0.33; 0.29]$, indicating no trade-off between visuocortical central cue and context processing.

Skin conductance responses

The ANOVA for mean skin conductance responses showed a significant main effect of context, $F(1, 39) = 20.65$, $p < .001$, $\eta_p^2 = .35$ $[0.15; 0.50]$, indicating potentiated skin conductance responses to both conditioned stimuli during the anxiety context (see Fig. 4.7). The main effect of cue was not significant, $F(1, 39) = 2.65$, $p = .112$, $\eta_p^2 = .06$ $[0.00; 0.21]$, however, there was also a trend for a Cue x Context interaction, $F(1, 39) = 3.09$, $p = .087$, $\eta_p^2 = .07$ $[0.00; 0.22]$, indicating enhanced differential processing during the neutral compared to the anxiety context.

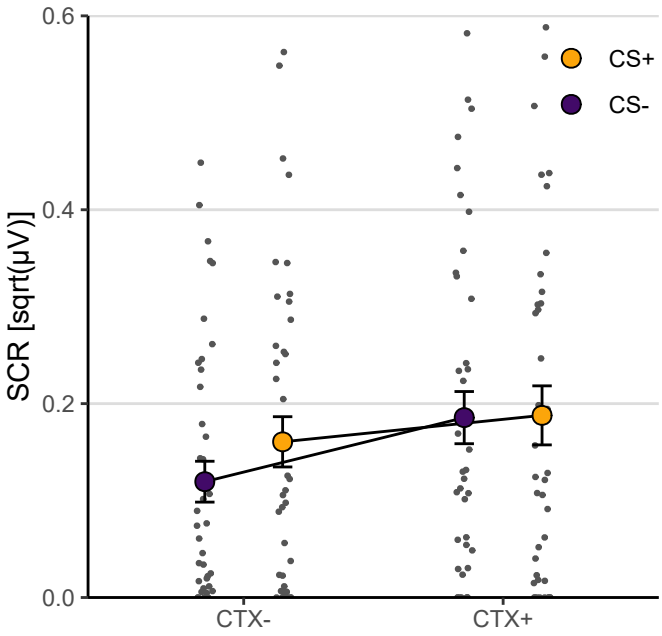

Figure 4.7: Mean skin conductance responses (±SEM) during the test phase. The anxiety context potentiated responses to both conditioned stimuli.

Aversive Ratings

Mean subjective responses to the contexts without concurrent presentations of the central cues revealed higher threat, $t(39) = -6.50$, $p < .001$, $d = -1.03$ $[-1.41; -0.64]$, and shock-expectancy ratings, $t(39) = -5.95$, $p < .001$, $d = -0.94$ $[-1.31; -0.56]$, for the anxiety compared to the neutral context, indicating successful induction of the threat-of-shock context. No difference was found for US-contingency ratings, $t(39) = -0.69$, $p = .493$, $d = -0.11$ $[-0.42; 0.20]$ (see Fig. 4.8).

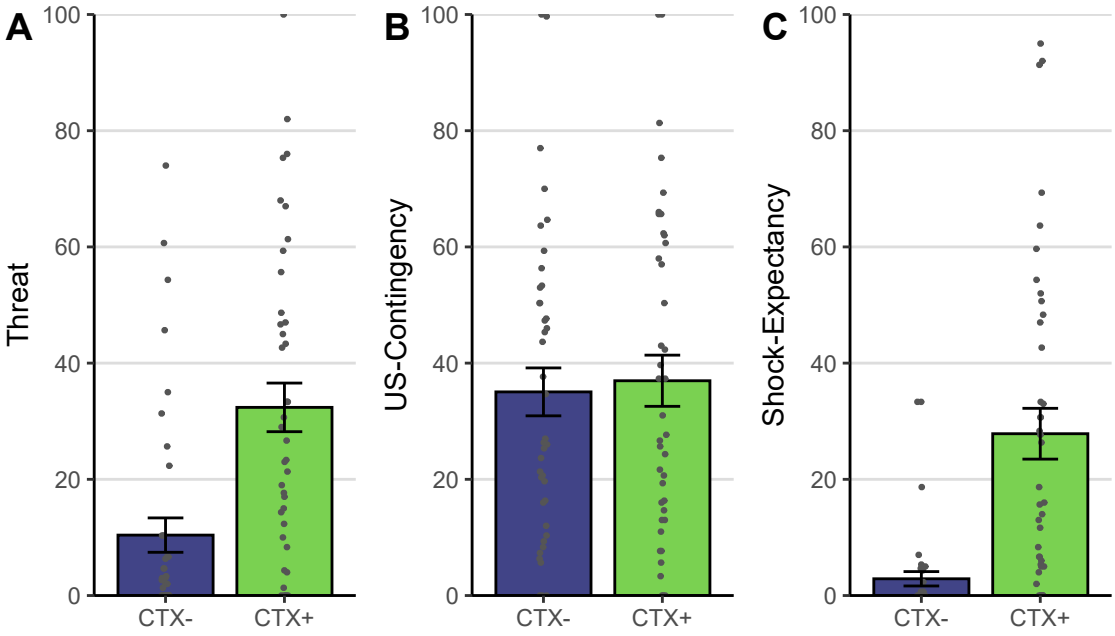

Figure 4.8: Mean subjective responses (±SEM) to the contexts without central cues during the test phase. (A) Subjective threat levels and (C) shock expectancy ratings are increased for the CTX+ compared to the CTX-, while there was no difference regarding US-contingency (B).

Analysis of subjective threat levels revealed a significant effect for cue, $F(1, 39) = 31.56$, $p < .001$, $\eta_p^2 = .45$ $[0.31; 0.63]$, and context, $F(1, 39) = 31.56$, $p < .001$, $\eta_p^2 = .45$ $[0.24; 0.58]$, indicating higher threat levels for the CS+ compared to the CS- and increased threat levels for both conditioned stimuli during the CTX+ as compared to during the CTX- (see Fig. 4.9 A). The cue x context interaction was not

significant, $F(1, 39) = 31.56$, $p < .001$, $\eta_p^2 = .45$ $[0.00; 0.14]$. The ANOVA for mean US-contingency ratings showed a significant effect of cue, $F(1, 39) = 66.21$, $p < .001$, $\eta_p^2 = .63$ $[0.46; 0.72]$, with higher US-contingency ratings for the CS+ than the CS-. The main effect of context, $F(1, 39) = 0.59$, $p = .449$, $\eta_p^2 = .01$ $[0.00; 0.12]$, and the cue x context interaction, $F(1, 39) = 1.60$, $p = .213$, $\eta_p^2 = .04$ $[0.00; 0.17]$, were not significant. For shock-expectancy ratings, the analysis revealed a significant effect of context, $F(1, 39) = 30.32$, $p < .001$, $\eta_p^2 = .44$ $[0.23; 0.57]$, indicating that participants expected the shock more during the CTX+ than during the CTX-. Furthermore, the main effect of cue was also significant, $F(1, 39) = 6.11$, $p = .018$, $\eta_p^2 = .14$ $[0.01; 0.30]$. Independent of the context, the participants expected the shock more during the CS+ than during the CS-. The interaction of cue and context did not reach significance, $F(1, 39) = 1.11$, $p = .299$, $\eta_p^2 = .03$ $[0.00; 0.15]$.

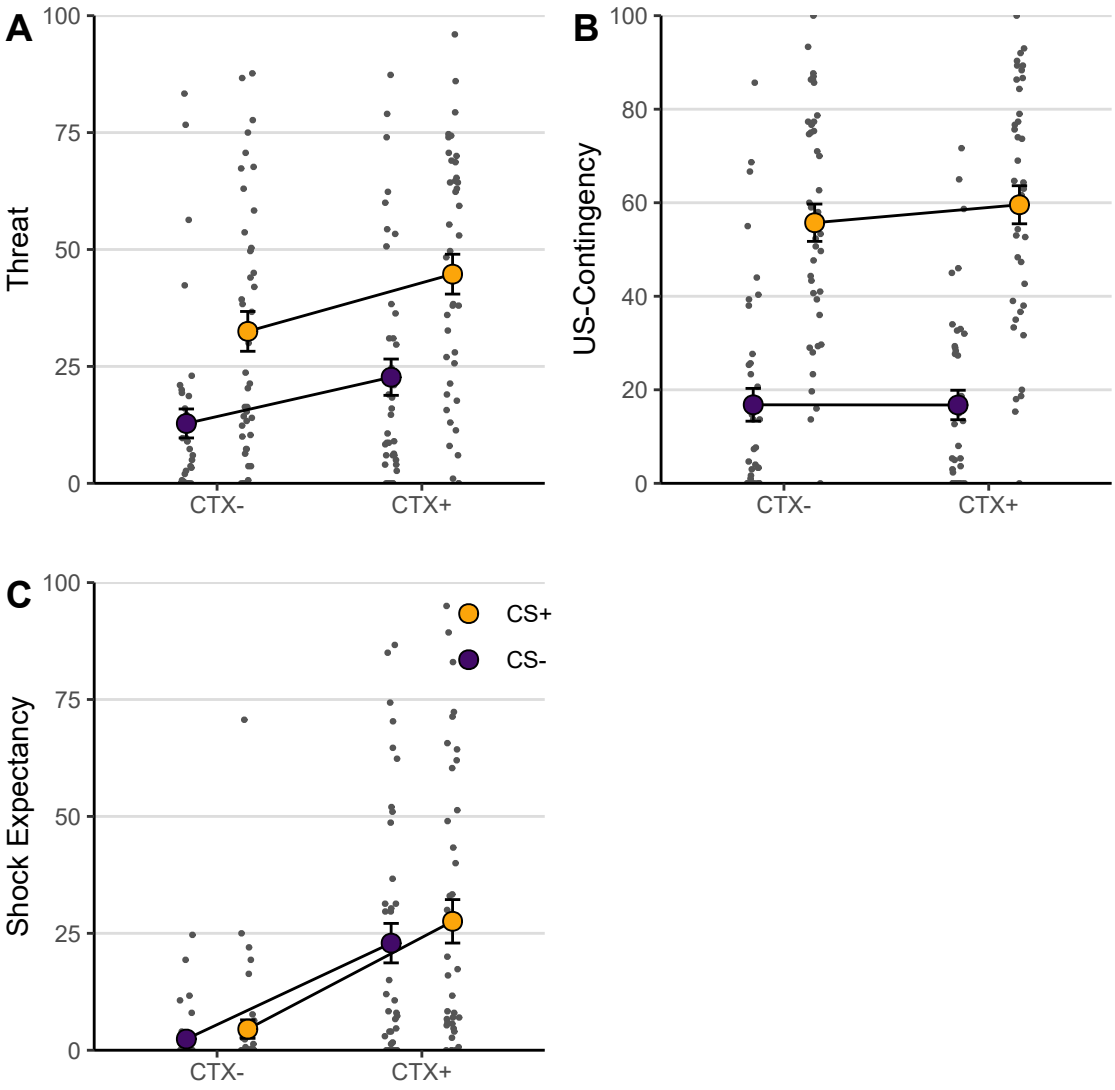

Figure 4.9: Mean subjective responses (±SEM) to the combination of central cue and context during the test phase. (A) Subjective threat levels and (C) shock expectancy ratings are increased for the CS+ compared to the CS- and for the CTX+ compared to the CTX-. (B) Mean US-contingency ratings show elevated responses for the CS+ compared to the CS- only.

4.4 Discussion

Combining fear conditioning with a threat-of-shock protocol, the presented study investigated the interplay of acute and potential threat and tested the hypothesis of

an additive interaction between fear and anxiety. This hypothesis was addressed using skin conductance responses and verbal reports of perceived threat and US-contingency as indices of defensive responding and steady-state visual evoked potentials to measure visuocortical activity.

During fear acquisition, results demonstrated successful cue conditioning: The CS+ was rated with higher mean threat and US-contingency ratings and elicited higher SCR amplitudes than the CS-. Subjective responses revealed also successful induction of anticipatory anxiety: The anxiety context was rated as more threatening and more associated with electrical stimuli than the neutral context, suggesting that participants expected potential threat following the verbal instruction (Bublatzky et al., 2010, 2014). Importantly, effects of aversive cue conditioning were stable throughout the threat-of-shock test phase. Independent of the context condition, CS+ presentations remained more threatening and more associated with an US than CS- presentations. In addition, during the neutral context, transient SCRs to the CS+ were enhanced compared to the CS-.

To quantify a potential interaction between fear and anxiety, the effect of the threat-of-shock context on cue conditioning was analyzed. Mean threat ratings demonstrated higher ratings to both central cues during the anxiety compared to the neutral context. Paralleling findings of Study 2, the effect of the anxiety context was not specific to the fear cue, but could be observed for both conditioned stimuli, indicating additive effects of fear and anxiety. On the other hand, the anxiety context had no impact on differential US-contingency (aversive noises) ratings. However, participants expected more electrical stimuli during the anxiety compared to the neutral context, and importantly, they also expected more electrical stimuli during the fear compared to the neutral central cue, even though the central cues were never paired -neither through experience nor verbal instruction- with the electrical stimuli. These results suggest that fear cues are more readily associated

with potential threat, indicating further evidence for a facilitation of fear responses during anxiety.

A very similar pattern could be obtained for skin conductance responses. The anxiety context increased transient SCRs to both central cues compared to the neutral context. Again, this effect was not selective to the fear cue, but generalized to the neutral cue as well. Actually, transient SCRs during the anxiety context did not differentiate between CS+ and CS- presentations. In addition, a marginal significant interaction between cue and context conditioning indicates that SCR amplitudes during the test phase were mainly driven by anticipatory anxiety. The reasons for this might be twofold: First, the anticipation of electrical stimuli during the anxiety context was more salient than the noise blasts at the end of the fear cue presentation. Participants were already confronted with 30 CS-US pairings during the fear acquisition phase, which might have resulted in a beginning habituation to the fear cues (Sperl et al., 2016). In contrast, participants could not familiarize themselves with the electrical stimuli (Bublatzky et al., 2014) and thus showed enhanced reactivity to the electrical stimuli compared to the noise blasts. Second, while anticipatory anxiety was instructed, fear acquisition was not. In the fear conditioning literature, it is well documented that non-instructed fear acquisition usually results in large variability between subjects regarding fear learning, with some participants learning the CS-US contingency and some not (Lonsdorf & Merz, 2017). In the present study, 14 out of 40 (=35%) individuals failed to show differential US-contingency ratings (greater than zero) at the end of fear acquisition, indicating that they did not learn the US-CS contingencies (e.g. Lonsdorf et al., 2019; Lonsdorf & Merz, 2017). Conversely, the verbal induction of anticipatory anxiety was more salient than the cue conditioning (see also Bublatzky, Guerra, & Alpers, 2018), potentially explaining why transient SCRs to the central cues were mainly driven by context effects.

In conclusion, measures of defensive responding consistently demonstrated en-

hanced fear responses during potential threat. These results replicate findings of Study 2 and substantiate the notion of functional interactions between fear and anxiety. Again, this interaction seems to be best characterized by additive mechanisms, however, results for SCR amplitudes indicate that these mechanisms are finely tuned to the motivational significance of the actual situation (Lang & Bradley, 2010) and therefore hint at a flexible deployment of defensive resources.

Regarding attentional mechanisms during acute threat, the present study yielded no compelling effects of cue conditioning during acquisition, even though there were significant differences for a small cluster of electrodes over the left-lateral occipital area (see Fig. 4.4). Higher ssVEP amplitudes for aversive compared to neutral cues would be in line with a growing body of research (Keil et al., 2013; Miskovic & Keil, 2013b), suggesting enhanced perceptual processing of fear-relevant signals (Miskovic & Keil, 2012). Again, this result might be influenced by the fact that more than one third of the participants in the present study did not learn the CS-US-contingency, potentially resulting in reduced differential ssVEP amplitudes.

Thus, it is not surprising that there was no effect of cue conditioning during the threat-of-shock test phase. The additional presentation of the contexts and the growing expectation of aversive electrical stimuli may have further interfered with visuocortical responding to the central stimuli. Equally, there was no effect of anxiety on the visuocortical responses to the contexts. On the contrary, results revealed that contexts in the neutral condition received facilitated sensory processing compared to the contexts in the anxiety condition, but only during presentations of the CS+. This result resembles previously reported findings by Wieser et al. (2016b), demonstrating enhanced visuocortical responses to the contexts during the presence of an acute threat-signaling stimulus. In another study, Wieser et al. (2014) found that centrally presented fear faces potentiated visuocortical responses to peripheral threatening scenes. In the actual study, however, this effect was evident for the neu-

tral context condition only, while contexts in the anxiety condition elicited reduced responses during the presence of fear cues. In combination with a visual trend for slightly enhanced responses to the central fear cue during the anxiety context, this finding suggests visuocortical competition, indicating that a reduction of ssVEP responses to the central cue is accompanied by an enhancement of ssVEP responses to the context and vice versa (Boylan et al., 2019). Importantly, visuocortical competition is expected to be enhanced as a function of motivational relevance. Consequently, competition should be enhanced for central fear cues and contexts in the anxiety condition compared to contexts in the neutral condition. However, a direct test of this hypothesis revealed no significant differences in visuocortical competition between conditions.

In conclusion, systematic research on how multiple, concurrent tasks influence visuocortical processing is lacking. Using frequency tagging allows the quantification of visuocortical processing of two or more overlapping and/or simultaneously presented visual stimuli (e.g. Wieser & Keil, 2014). Associating these stimuli with different tasks further enables the investigation of their visual competition or attentional prioritization. Yet it remains an open question, how these tasks impact each other and to which extent they are influenced by lower-level properties of the visual stimuli (like size, color or orientation) and higher-level task-specific or emotional features. Recent studies have begun to elucidate the visuocortical competition between different concurrent tasks. For example, Boylan and colleagues (2019) found no interference between visuocortical responses to threat-related features and a concurrent feature selection task. Wieser et al. (2014) demonstrated that viewing fearful facial expressions selectively amplifies ssVEP responses to contextual threat. However, no study has yet investigated visuocortical responding during two concurrent aversive learning tasks. Future studies need to fill this gap by systematically exploring interferences between different threat- and task-related features.

It is also important to mention that this study used verbal instructions to induce potentially threatening contexts, i.e. participants consciously expected aversive events during the anxiety, but not during the neutral context. However, the extent to which ssVEP responses during aversive learning are shaped by expectancy is still an open question. First studies demonstrated that conscious expectancy of aversive stimuli is not sufficient to enhance visuocortical activity (Moratti & Keil, 2009; Yuan et al., 2018) and emphasize the importance of actually experiencing aversive outcomes to initialize changes in visuocortical areas (Miskovic & Keil, 2012).

Taken together, while measures of defensive responding demonstrated successful cue and context conditioning and yielded further evidence for additive interactions between fear and anxiety, no changes in visuocortical activity as indices of threat detection mechanisms could be observed. In order to investigate visuocortical changes during orthogonally combined cue and context conditioning, more research on attentional processes during competing tasks is warranted, especially with two or more tasks relying on similar aversive learning mechanisms. Finally, the present study conceptually replicated the main findings of Study 2 and substantiated the notion of an additive model of fear and anxiety. Having established a laboratory paradigm to investigate interactions of threat processing mechanisms during acute and potential threat, the intriguing question arises, how these interactions are expressed in the neural networks of fear and anxiety. Given the well characterized neural circuits underlying fear and anxiety (Alvarez et al., 2011; Davis et al., 2010; Tovote et al., 2015), future studies should adapt the present paradigm for fMRI-methods in order to elucidate the interaction between fear and anxiety on a neural level.

Study 4: Investigating sustained attention in contextual anxiety using steady state VEPs evoked by flickering video stimuli

5.1 Introduction

Over the last decade, context conditioning has been established as a laboratory model for potential threat (Davis et al., 2010; Glotzbach et al., 2012). During context conditioning, aversive events are administered unpredictably in one out of two contexts, while the other context remains unpaired. In contrast to cued fear conditioning, there are no threat-predicting signals, making the context the next best predictor for the occurrence of aversive events. The absence of discrete threat-signaling stimuli results in a sustained state of anxious apprehension (Grillon et al., 2004).

In rodent studies, the immediate surroundings -usually the test cages- serve as context stimuli (Haaker et al., 2019). To translate findings from animal to human research, different physical test rooms have been used to establish distinct contexts in human fear conditioning studies (e.g. Klinke, Fiedler, Lange, & Andreatta, 2020). However, it is not always feasible nor possible to implement context stimuli through physical rooms. Therefore, basic research frequently relied on less complex and more controllable stimuli, like different background colors (Lang et al., 2009; Vansteenwegen et al., 2008), geometrical symbols (e.g. Study 1-3 and Wieser et al., 2016b), or colored picture frames (Bublatzky et al., 2014), which were presented on monitor screens.

These stimuli can be defined as contexts, because they are presented for a sustained duration, comprise other discrete events and set a cognitive context for the upcoming task or expected outcome (Maren et al., 2013). In addition, using simple visual stimuli as contexts enables precise timing of the on- and offsets and facilitates comparability between different contexts. On the other hand, those stimuli often lack ecological validity, as in real life, contexts are encoded as conjunctive representations of multiple elements (e.g. Genheimer, Andreatta, & Pauli, 2020) and individuals are typically able to freely explore the space (Glotzbach et al., 2012).

In order to overcome these issues, Virtual Reality (VR) provides an optimal tool to bridge the gap between simple, but controllable and complex, but ecologically valid contexts. VR can be used to create complex and enriched environments and at the same time, it enables high control over the timing and comparability of context stimuli. For example, Andreatta et al. (2020) used virtual reality to create two different virtual offices that were similar regarding floor plan, size, and complexity, but differed in the arrangement of the furniture. Moreover, the authors implemented three additional rooms, which shared physical properties and furniture of both rooms to a specific proportion, to investigate generalization of contextual anxiety. Using appropriate methods, like motion-tracking or via joystick, individuals are also able to freely navigate through the contexts in virtual reality (Andreatta et al., 2015; Glotzbach et al., 2012). Yet, participants remain stationary in order to record periphysiological parameters (e.g. Glotzbach-Schoon, Andreatta, et al., 2013). Consequently, VR is well suited to investigate context conditioning in highly controlled laboratory settings.

Recent studies used these and similar virtual environments to investigate responding to potential threat. They found unanimous evidence for higher ratings of subjective anxiety (Andreatta et al., 2015; Glotzbach et al., 2012), potentiated startle-reflexes (Glotzbach-Schoon, Andreatta, et al., 2013), elevated skin conductance levels (Glotzbach-Schoon, Tadda, et al., 2013) and increased avoidance be-

havior (Glotzbach et al., 2012) during the conditioned versus the neutral context. In addition, Glotzbach-Schoon, Tadda, et al. (2013) could demonstrate that verbal and physiological responding to contextual anxiety increases as a function of individual trait-anxiety. Andreatta and colleagues (2015) also utilized virtual reality to elucidate neural activity during context conditioning and extinction learning. In a first phase, participants could freely explore two different virtual offices to familiarize themselves with the virtual reality and to establish a spatial map of the contexts. Context conditioning and extinction learning was then tested in fMRI. Participants were passively guided through the virtual offices on pre-recorded paths, while they received electrical stimulation in one, but never in the other office. Besides successful conditioning, results revealed different neural activity during the onset compared to later intervals of the anxiety context. Increased initial responses to the anxiety compared to the neutral context were found in the primary motor cortex and frontal brain regions, including orbitofrontal (OFC), dorsolateral (dlPFC) and dorsomedial (dmPFC) prefrontal cortex, suggesting conscious awareness of threat contingencies and explicit threat appraisal (Andreatta, Glotzbach-Schoon, et al., 2015). Sustained responses could be identified in the amygdala and hippocampus, indicating enhanced involvement of the fear/anxiety-network (amygdala) and neural representations of the spatial map of the context rooms (hippocampus). Strikingly, these results demonstrate that context conditioning is a complex construct characterized by dynamic involvement of multiple response systems over time.

Regarding attentional processes, potential threat induced by context conditioning prompts heightened vigilance (Lang et al., 2000). The function of hypervigilance during anxiety involves monitoring for potential threat by broadening attention and increases scanning of the context in order to facilitate threat detection (Richards et al., 2014). Evidence for this hypothesis mainly stems from studies using visual search paradigms (e.g. Bar-Haim et al., 2007) or eye-tracking studies (Richards, Hadwin,

Benson, Wenger, & Donnelly, 2011; Wieser, Pauli, Weyers, Alpers, & Mühlberger, 2009). However, the neural mechanics underlying heightened vigilance to threat remain unclear (Richards et al., 2014).

To this end, a recent study investigated steady-state visual evoked potentials as direct neurophysiological marker of visual attention during context conditioning (Kastner et al., 2015). The authors used screenshots of the above-mentioned virtual offices to implement two different contexts. Importantly, the full screenshot was presented in flickering mode to induce ssVEPs. Results indeed revealed heightened ssVEP amplitudes throughout the whole 20 s presentation of the anxiety compared to the neutral context, suggesting cortical facilitation of perceptual processing during the anxiety context as a visuocortical correlate of hypervigilance.

Having demonstrated the feasibility of recording ssVEPs to index sustained attention to static images during context conditioning, the next crucial step is to quantify visuocortical responding to more ecologically valid context stimuli. Therefore, the present study utilized video stimuli of virtual offices to implement differential context conditioning. Similar to Andreatta, Glotzbach-Schoon, et al. (2015), participants were passively guided through the offices in order to establish spatial representations of the contexts. The two main goals of this study are to 1) successfully induce ssVEPs using video stimuli and to 2) investigate changes in visuocortical responding during potentially threatening contexts and successive extinction learning.

5.2 Methods and material

5.2.1 Sample

In total forty subjects participated in the experiment, of which two were excluded due to data recording failures during the experiment. The final sample included 38 participants (24 females, mean age ± SD: 23.63 ± 3.72 years). Participants were

required to be between 18 and 35 years old, free of any family history of photic epilepsy, any mental or neurological disorders, and have normal or corrected vision. Participants completed the German versions of Spielberger's State-And-Trait Anxiety Inventory (STAI; Laux & Spielberger, 1981), the Anxiety Sensitivity Index 3 (ASI-3; Reiss et al., 1986; Taylor et al., 2007), the Intolerance of Uncertainty Scale (IUS; Buhr & Dugas, 2002), the GAD-7 questionnaire (Spitzer et al., 2006), and the Depression-Anxiety-Stress-Scale (DASS; Lovibond & Lovibond, 1995). For a summary of the questionnaire scores see Table 5.1. All participants gave written informed consent and were paid 15 € or received an equivalent in course credits. All procedures were approved by the ethics committee of the University of Würzburg.

5.2.2 Stimuli and Apparatus

Videos recorded from virtual reality served as context stimuli. The virtual environment was created with Valve Corporation's Source Engine (Bellevue, USA) and has been successfully used in other context conditioning studies (e.g. Andreatta et al., 2019). During each video, the participant started in a corridor in front of one of two different office rooms. After the door opened, the participant was passively guided through the office on a pre-recorded pathway. After about 35 seconds, the participant left the office room and the video ended. There was one pathway in the clockwise and one in the counterclockwise direction per room. The two virtual offices were designed to be similar regarding size, floor and lighting and only differed in furniture arrangement, window style and decoration. Video stimuli were counter-balanced for conditions (CTX+ vs. CTX-) across participants. All stimuli were presented on a 19-inch monitor (resolution = 1024 x 768 pixels) with a vertical refresh rate of 60 Hz, located ca. 100 cm in front of the participant, using the Presentation software (Neurobehavioral Systems, Inc., Albany, CA, USA). The videos stimuli spanned a visual angle of 14.75° horizontally and 11.14° vertically. In order to evoke steady-state

potentials, video stimuli were presented in flickering mode in 20 Hz.

Table 5.1: Descriptive statistics of the questionnaire data

	N	M	SD	Mdn	Min	Max
Age	38	23.63	3.72	23.00	18	34
STAI-S	38	35.97	9.22	34.50	20	63
STAI-T	38	36.84	9.69	35.00	20	59
ASI-3	38	16.11	10.83	12.50	2	39
IUS	38	44.58	11.62	45.00	23	67
GAD-7	38	4.45	3.17	4.00	0	13
DASS-D	38	3.42	3.09	2.50	0	12
DASS-A	38	2.18	1.81	2.00	0	6
DASS-S	38	5.68	3.94	5.00	0	17
US-Intensity	38	1.89	1.02	1.69	1	6
US-Unpleasantness	38	5.32	1.04	5.00	4	8

Note: STAI = State-Trait-Anxiety-Inventory (S: Subscale State, T: Subscale Trait); ASI-3 = Anxiety Sensitivity Index 3; IUS = Intolerance of Uncertainty Scale; DASS = Depression-Anxiety-Stress-Scale (D: Subscale Depression, A: Subscale Anxiety, S: Subscale Stress); US-Intensity in mA.

Aversive unconditioned stimuli (US) were 20 ms electric pulse trains (2 ms pulse width, 25 Hz), which were delivered to the left calf through surface bar electrodes consisting of two gold-plated stainless-steel disks of 9 mm diameter and 30 mm spacing. The electric stimuli were generated by a constant current stimulator (Digitimer DS7A, Digitmer Ltd., Welwyn Garden City, UK). Prior to the actual experiment, the US intensity was adjusted to the individual pain-threshold. Thus, participants received two series of increasing and decreasing intensities until they reached a level

they described as "just noticeable pain" – corresponding to 4 on a scale from 0 (no pain at all) to 10 (unbearable pain). The individual US intensity was determined by calculating the mean of the four series' final intensities and then adding 30% to avoid habituation. The resulting intensities and subjective pain ratings were 1.89 ± 1.02 mA (mean intensity ± SD) and 5.32 ± 1.04 (mean pain rating ± SD).

5.2.3 Procedure

Participants were seated in a sound-attenuated, dimly lit testing room, where EDA-electrodes and the EEG-net were applied. The study consisted of a pre-acquisition, acquisition and extinction phase. During pre-acquisition each video was presented once (2 videos per room) and no US was delivered (see Fig. 5.1). The ITI had a random duration between 8 s and 10 s. Pre-acquisition was followed by two rating trials, which started as normal trials, but were paused after about 10 s, in which a visual analog scale was presented to collect online ratings. During this procedure, participants were asked to rate the current room regarding valence, arousal (both 9-point Likert-scales; from 1 = very unpleasant/ very calm to 9 = very pleasant/ very exciting), anxiety and US-expectancy (both visual analogue scales from 0 = not anxious/ not likely to 100 = very anxious/ very likely). As soon as the participant finished the rating procedure, the video continued. Rating trials were excluded from physiological and electrocortical analysis. Acquisition consisted of 16 video trials (8x CTX+ and 8x CTX-) plus four additional rating trials after the first half and in the end of the phase. US-delivery in the CTX+ started after 7 s and was omitted after 32 s to ensure that participants received US only inside of the office room. Per CTX+, zero to three US were unpredictably delivered with an interval of at least 5 s between two US. US were also presented during CTX+ rating trials, resulting in a total of 15 US presentations. Importantly, at a random timepoint during the last half of each trial an interval of 6 s was implemented, in which no US was delivered. This

interval was later used for EEG-analysis without confounding US-presentations. The extinction phase was identical to acquisition regarding trials and timing, but no US were delivered. Participants were instructed to reduce eye-movements and focus on a fixation cross that was centrally presented throughout the experiment.

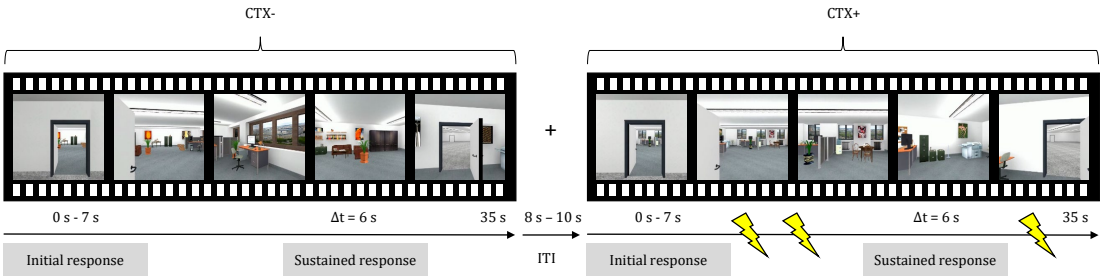

Figure 5.1: Experimental Design. Participants watched videos, in which they were guided through one of two different virtual offices. In one office (anxiety context; CTX+), 0 to 3 US were presented unpredictably. No US were presented in the other office (safety context; CTX-). Videos were presented in 20 Hz flicker frequency to evoked ssVEPs. To analyze ssVEPs and skin conductance responses during the initial response window, no US was presented during the first 7 s after video onset. At a random timepoint during the second half of each video, US-delivery was omitted for an interval of 6 s to analyze sustained ssVEP responses.

5.2.4 Physiological data processing

Skin conductance was recorded using two silver-silver chloride electrodes placed on the thenar and hypothenar eminences of the participants' non-dominant palmar surface. The signal was recorded with a V-Amp amplifier and Vision Recorder Software (BrainProducts Inc., Munich, Germany). A sampling rate of 1,000 Hz and a notch-filter at 50 Hz were applied. Analysis was then performed using Vision Analyzer Software (BrainProducts Inc., Munich, Germany). For each experimental condition, the trough-to-peak value within 1 s to 6 s after video-stimulus onset was scored manually, square-root-transformed and then divided by the participant's maximum SCR to a context onset. SCRs smaller than 0.02 µS were scored as zero responses before

transformation.

5.2.5 EEG recording and data processing

EEG data analysis was conducted and is reported according to published guidelines51. Electrocortical brain activity was recorded using a 129 electrodes Electrical Geodesics System (EGI, Eugene, OR) referenced to the vertex electrode (Cz), with a sampling rate of 250 Hz and an on-line band-pass filter of 0.1-100 Hz. Electrode impedances were kept below 50 kΩ. Subsequent data processing occurred off-line using the EMEGS software for Matlab52. In a first step, all data were filtered using a 40-Hz low-pass filter (cut-off at 3 dB point; 45 dB/octave, 19th order Butterworth), before extracting epochs from 600 ms pre- to 6,900 ms post-onset for the initial response and from 400 ms to 5,900 ms during the late interval for the sustained electrocortical processing. Following the guidelines for the statistical correction of artefacts in dense array studies procedure (SCADS; Junghofer et al., 2000), we first detected individual channel artefacts based on the original recording reference (Cz), before data were re-recorded to the average reference to identify global artefacts. Bad sensors within individual trials were identified based on rejection criteria for the distributions of the maximum absolute amplitude, standard deviation and gradient. Contaminated trials were removed, if they included more than 20 bad sensors. After rejection, contaminated sensors of the remaining epochs were interpolated using weighted spherical splines fit to all remaining sensors. The retention rate for initial and sustained responses were 64.5 ± 23.5 % and 78.7 ± 20.0 % ($M \pm SD$), respectively. Remaining epochs were averaged separately for the two context conditions and the three main phases of the experiment. To reduce the impact of volume conductance, the current source densities (CSD) of the time-averaged data were calculated. The CSD transformed data were then submitted to a Fast-Fourier-algorithm on a time interval between 2000 and 6500 ms post-onset for the initial response and from 1400 ms to

5,900 ms during the sustained response interval. The first 2000 ms after stimulus onset were omitted since the virtual door to the office opens between 1000 and 2000 ms after stimulus onset. The time window of the sustained response interval was chosen to be the same in total length as the initial response interval to facilitate comparability between initial and sustained responses.

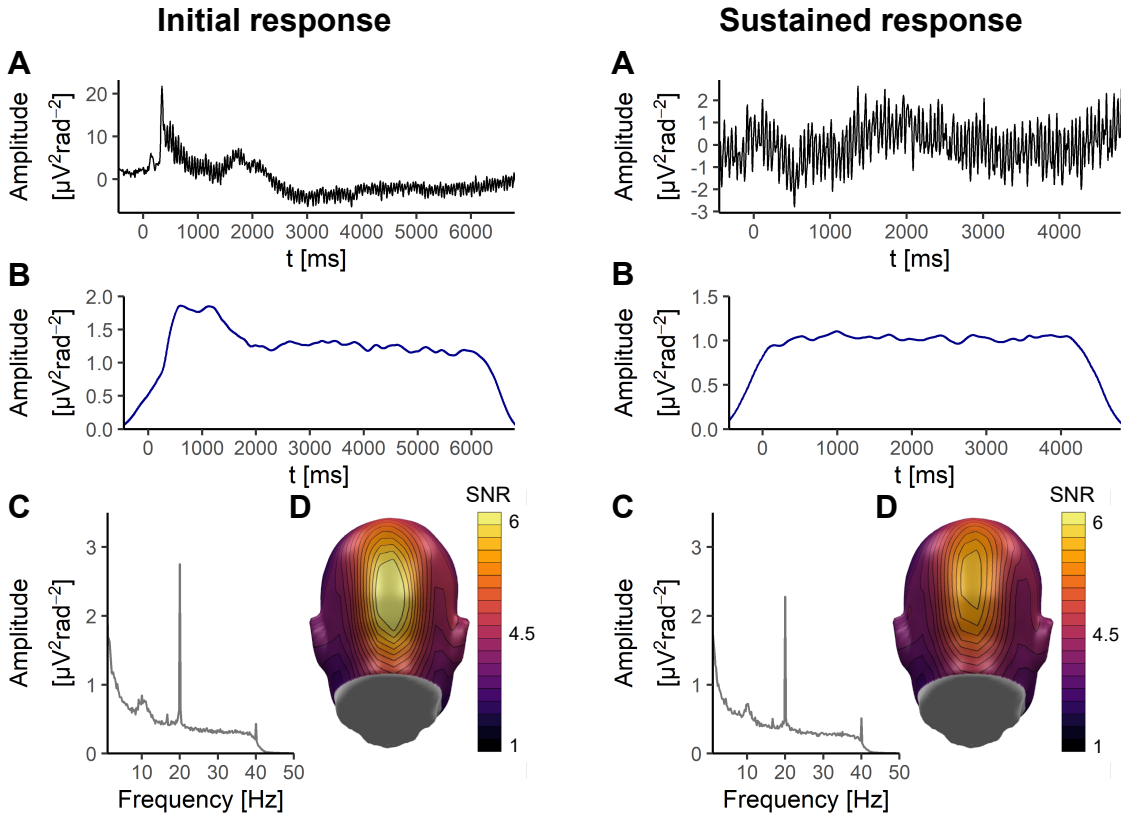

Figure 5.2: Characteristics of the grand averaged ssVEP signal during the initial and sustained response window across all participants and conditions at Oz (sensor 75): (A) Time-domain representation of the CSD-transformed ssVEP response. (B) Time-frequency analysis of the Hilbert-transformed 20 Hz driving frequency. (C) Frequency-domain representation. (D) Topographies of the signal-to-noise ratio.

In a next step, we obtained the signal-to-noise ratio (SNR) for the driving frequency by dividing the power of the 20 Hz frequency by the mean of the spectral power at six adjacent frequency bins, leaving out the two immediate neighbors. The SNR is a unitless measure that accounts for both the evoked signal and the ran-

dom noise in the data and has recently been used in other ssVEP paradigms as well (Barry-Anwar et al., 2018; Boylan et al., 2019). The CSD-transformed ssVEP signals for a representative electrode (Oz), the Fast-Fourier-Transformation on these ssVEPs, the time-frequency representations of the driving frequencies, and the topography of their SNRs averaged across all subjects and conditions are shown in Fig. 5.2. For statistical analysis, the ssVEP activity was pooled across the Oz and 7 surrounding electrodes (EGI sensors 70, 71, 72, 74, 75, 76, 82, 83; Wieser et al. (2014a))

5.2.6 Statistical Analyses

The mean SCR to context onset and the mean ssVEP amplitudes during the initial and sustained response window were analyzed separately with mixed-measure analysis of variances (ANOVA) with the within-subject factors context (CTX+ vs CTX-) and phase (Acquisition vs Extinction). The same procedure was carried out for valence, arousal, anxiety and US-expectancy ratings. Differences in the pre-acquisition phase of the experiment were analyzed with simple t-tests. Significant effects were followed up using ANOVAs and t-tests where appropriate. A significance level of 0.05 was used for all analyses and Greenhouse-Geisser correction was applied where appropriate (Greenhouse & Geisser, 1959). Throughout this manuscript, the partial η^2 ($\eta_p{}^2$) or Cohen's d (d) and their 95% confidence interval are reported as standardized effect sizes.

5.3 Results

5.3.1 Pre-Acquisition Phase

During pre-acquisition, CTX+ and CTX- did not differ regarding valence, $t(37) = -0.42$, $p = .675$, $d = -0.07$ $[-0.39; 0.25]$, anxiety, $t(37) = -1.56$, $p = .127$,

$d = -0.25$ $[-0.57; 0.07]$, skin conductance responses, $t(33) = 0.22$, $p = .826$, $d = 0.04$ $[-0.30; 0.37]$, and ssVEP amplitudes during the initial, $t(31) = 0.25$, $p = .806$, $d = 0.04$ $[-0.30; 0.39]$, or the sustained response window, $t(37) = 1.70$, $p = .098$, $d = 0.28$ $[-0.05; 0.60]$ (see Fig. 5.3). Surprisingly, there was a significant difference between CTX+ and CTX- for arousal ratings, $t(37) = -0.11$, $p = .917$, $d = -0.02$ $[-0.33; 0.30]$, indicating higher arousal ratings for CTX+ compared to CTX-, although no US had been delivered yet. However, this difference is only present for arousal ratings and video stimuli were counterbalanced across participants, so this finding is most likely a false-positive.

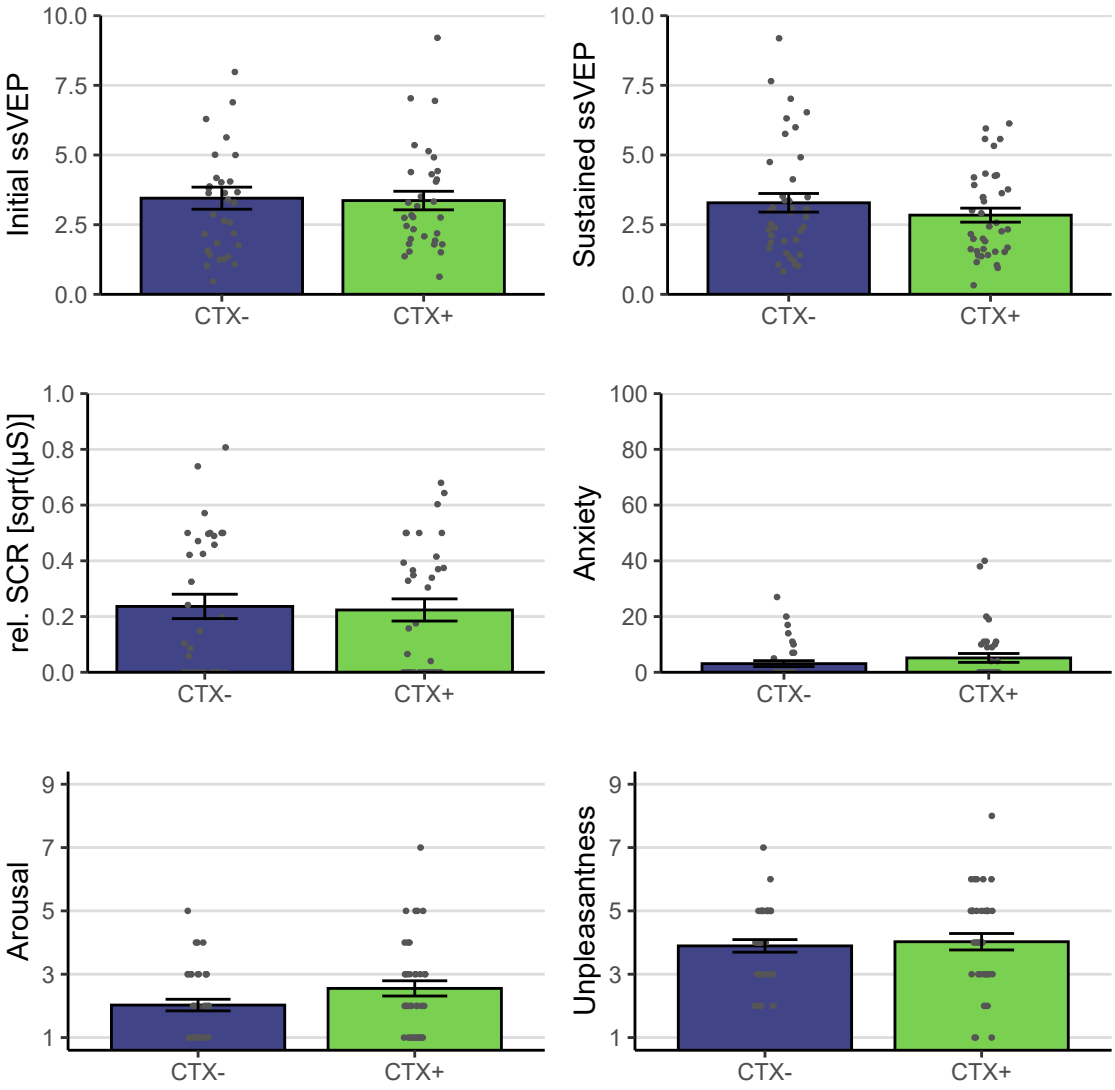

Figure 5.3: Mean defensive responses (±SEM) during the pre-acquisition phase.

5.3.2 Acquisition and Extinction Phase

Steady-state visual evoked potentials

Regarding initial response window, there was neither a significant main effect of context, $F(1, 37) = 0.03$, $p = .857$, $\eta_p^2 < .01$ [0.00; 0.05], phase, $F(1, 37) = 2.87$, $p = .099$, $\eta_p^2 = .07$ [0.00; 0.22], nor a Context x Phase interaction, $F(1, 37) = 0.18$, $p = .674$, $\eta_p^2 < .01$ [0.00; 0.09] (see Fig. 5.4).

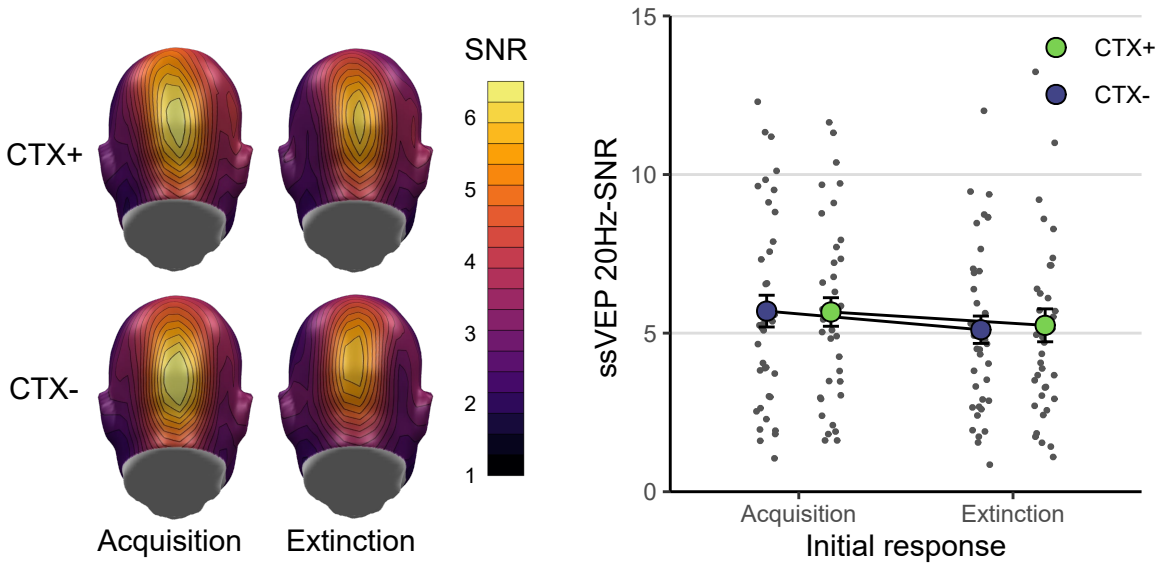

Figure 5.4: Topographies (left) and the corresponding mean (±SEM) visuocortical responses (right) to the context cues during the initial response window.

During the sustained response window, there was a significant main effect of context, $F(1, 37) = 30.64$, $p < .001$, $\eta_p^2 = .45$ [0.24; 0.59], and phase, $F(1, 37) = 18.42$, $p < .001$, $\eta_p^2 = .33$ [0.13; 0.49], which were further qualified by a significant Context x Phase interaction, $F(1, 37) = 41.73$, $p < .001$, $\eta_p^2 = .53$ [0.33; 0.65]. Post-hoc t-tests indicated stronger visuocortical engagement to the CTX- compared to the CTX+, $t(37) = 9.06$, $p < .001$, $d = 1.47$ [1.00; 1.93], during acquisition but not during extinction, $t(37) = -0.18$, $p = .862$, $d = -0.03$ [−0.35; 0.29] (see Fig. 5.5).

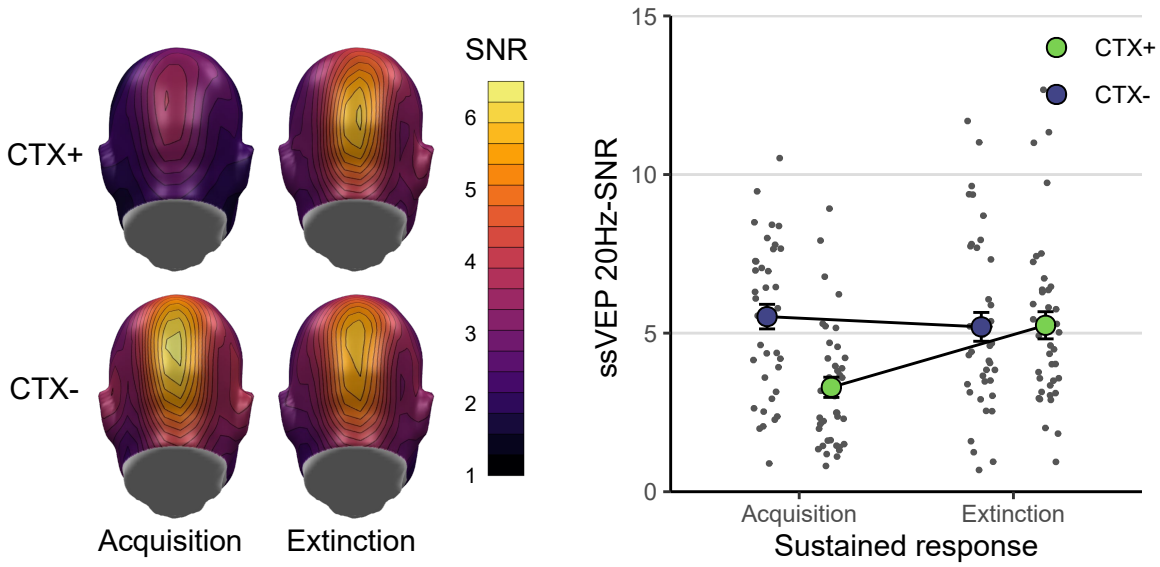

Figure 5.5: Topographies (left) and the corresponding mean (±SEM) visuocortical responses (right) to the contexts during the sustained response window.

To test the hypothesis that the increased visuocortical engagement to the CTX- compared to the CTX+ was due to electrical stimuli that were presented prior to the sustained response interval, the ssVEP data was reanalyzed excluding any trials in which electrical stimuli were presented within 5,000 ms before the sustained response interval. Consequently, 2.94 ± 1.41 (*mean* ± *SD*; *range* = [0,5]) trials per participant were removed from the analysis. However, the differences between CTX+ and CTX- in the remaining trials remained significant, $t(37) = 7.20$, $p < .001$, $d = 1.17$ $[0.75; 1.58]$, suggesting that the electrical stimulation did not decrease the visuocortical engagement to the CTX+. For a more detailed exploratory analysis, CTX+ trials were subdivided into four categories according to the total number of US (0 - 3) presented prior to the sustained response interval (see Fig. 5.6A). Interestingly, ssVEP amplitudes were marginally significantly higher for the CTX+ trials without US compared to the CTX+ trials with one US prior to the response window, $t(33) = 1.95$, $p = .059$, $d = 0.33$ $[-0.01; 0.68]$, while there were no differences among the reminder of the CTX+ trials, all $ps > .259$. Critically, all CTX+ trials, especially

CTX+ trials without preceding US presentations elicited significantly smaller ssVEP amplitudes than CTX- trials [0 US: $t(36) = 7.11$, $p < .001$, $d = 1.17$ [0.74; 1.58]; 1 US: $t(34) = 9.34$, $p < .001$, $d = 1.58$ [1.07; 2.07]; 2 US: $t(24) = 5.75$, $p < .001$, $d = 1.15$ [0.63; 1.65]; 3 US: $t(11) = 3.08$, $p = .011$, $d = 0.89$ [0.20; 1.55]].

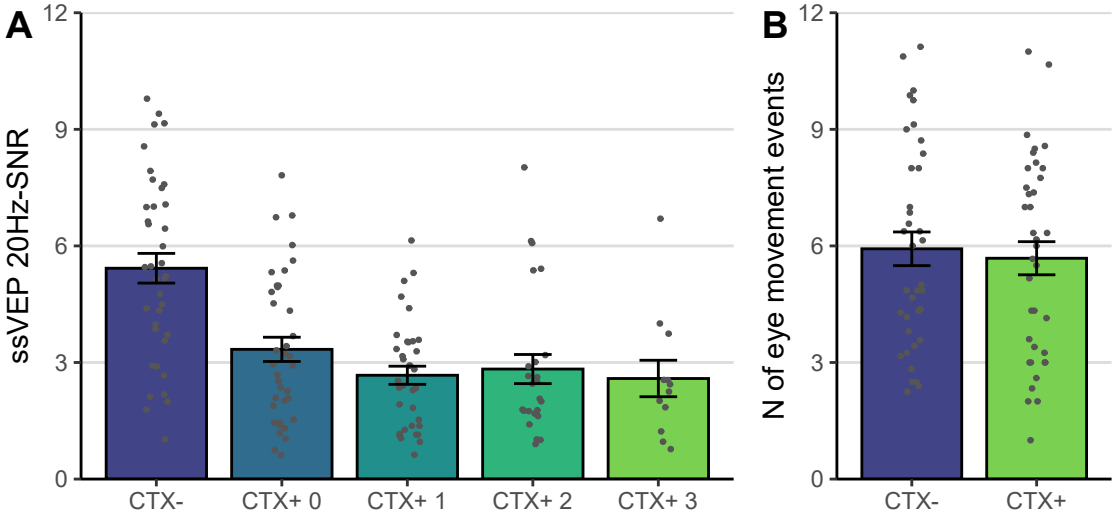

Figure 5.6: Post-hoc analyses of the ssVEP and EOG signal during the sustained response window. (A) Mean visuocortical responses (±SEM) during the CTX- and CTX+ as a function of the total number of preceding US, ranging from 0 (CTX+ 0) to 3 (CTX+ 3). (B) Mean number of eye-movement events (±SEM) as indexed by substantial EOG signal changes during the CTX+ and CTX-

Another explanation for the decreased responses to the CTX+ is that there was more eye movement during the anxiety context, which might have led to a disruption of the ssVEP signal. To quantify eye-movement, we calculated the Euclidean norm of the signals recorded by the EOG electrodes measuring horizontal and vertical eye-movements ($\sqrt{EOG_H^2 + EOG_V^2}$) on single trial level. Eye-movement events were defined as samplepoints, where the difference of the normed EOG signal and the subject mean exceeded three standard deviations. The minimum interval between two eye-movement events was set to 50 ms. Comparing the mean number of eye-movement events between CTX+ and CTX- (see Fig. 5.6B) yielded no significant

differences, $t(36) = 0.59$, $p = .560$, $d = 0.10$ $[-0.23; 0.42]$. Eye-movement as indexed by the EOG signal did not differ between conditions, and consequently, might not have had an impact on the ssVEP signal.

Skin conductance responses

Four participants were removed from SCR analysis because they showed no quantifiable skin conductance response. The ANOVA for the remaining participants showed no significant main effect of phase, $F(1, 33) = 1.29$, $p = .263$, $\eta_p^2 = .04$ $[0.00; 0.18]$, or phase x context interaction, $F(1, 33) = 0.01$, $p = .939$, $\eta_p^2 < .01$ $[0.00; 0.01]$. The main effect of context, $F(1, 33) = 3.78$, $p = .060$, $\eta_p^2 = .10$ $[0.00; 0.27]$ was marginally significant. However, post-hoc t-tests revealed no significant differences between the CTX+ and CTX- during acquisition, $t(33) = -1.55$, $p = .130$, $d = -0.27$ $[-0.61; 0.08]$, or during extinction, $t(33) = -1.48$, $p = .147$, $d = -0.25$ $[-0.59; 0.09]$ (see Fig. 5.7).

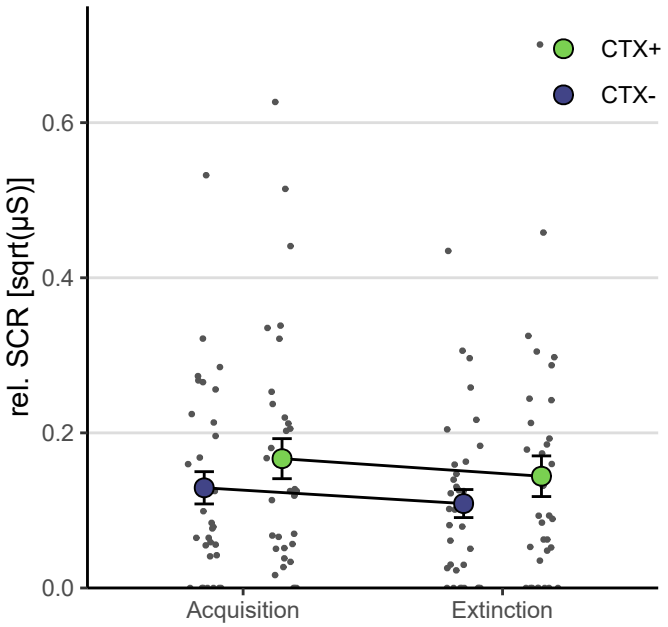

Figure 5.7: Mean skin conductance responses (±SEM) to video stimulus onsets

Ratings of the contexts

Phase x context ANOVAs revealed significant main effects of phase (all $ps < 0.001$) and context (all $ps < 0.001$), which were further qualified by phase x context interactions for valence, $F(1, 37) = 19.27$, $p < .001$, $\eta_p^2 = .34$ $[0.14; 0.50]$, arousal, $F(1, 37) = 28.04$, $p < .001$, $\eta_p^2 = .43$ $[0.22; 0.57]$, anxiety, $F(1, 37) = 10.57$, $p = .002$, $\eta_p^2 = .22$ $[0.05; 0.39]$, and US-expectancy ratings, $F(1, 37) = 41.93$, $p < .001$, $\eta_p^2 = .53$ $[0.33; 0.65]$. During acquisition, participants rated the CTX+ as more unpleasant, $t(37) = -7.67$, $p < .001$, $d = -1.24$ $[-1.66; -0.81]$, with higher emotional arousal, $t(37) = -8.78$, $p < .001$, $d = -1.42$ $[-1.87; -0.97]$, more anxiogenic, $t(37) = -7.09$, $p < .001$, $d = -1.15$ $[-1.56; -0.73]$, and more associated with an US, $t(37) = -11.71$, $p < .001$, $d = -1.90$ $[-2.43; -1.36]$, than the CTX- (see Fig. 5.8). During extinction, ratings to the CTX+ decreased [Arousal: $t(37) = 6.39$, $p < .001$, $d = 1.04$ $[0.64; 1.43]$; Unpleasantness: $t(37) = 5.96$, $p < .001$, $d = 0.97$ $[0.58; 1.35]$; Anxiety: $t(37) = 4.15$, $p < .001$, $d = 0.67$ $[0.32; 1.02]$; US-Expectancy: $t(37) = 7.99$, $p < .001$, $d = 1.30$ $[0.86; 1.72]$], while ratings to the CTX- did not change [Arousal: $t(37) = 1.21$, $p = .234$, $d = 0.20$ $[-0.13; 0.52]$; Unpleasantness: $t(37) = -1.86$, $p = .071$, $d = -0.30$ $[-0.62; 0.03]$; Anxiety: $t(37) = 1.83$, $p = .075$, $d = 0.30$ $[-0.03; 0.62]$; US-Expectancy: $t(37) = 0.63$, $p = .532$, $d = 0.10$ $[-0.22; 0.42]$]. At the end of extinction, however, there were still significant differences between CTX+ and CTX- for arousal, $t(37) = -5.50$, $p < .001$, $d = -0.89$ $[-1.26; -0.51]$, unpleasantness, $t(37) = -5.27$, $p < .001$, $d = -0.85$ $[-1.22; -0.48]$, anxiety, $t(37) = -5.55$, $p < .001$, $d = -0.90$ $[-1.27; -0.52]$, and US-expectancy ratings, $t(37) = -7.27$, $p < .001$, $d = -1.18$ $[-1.59; -0.76]$.

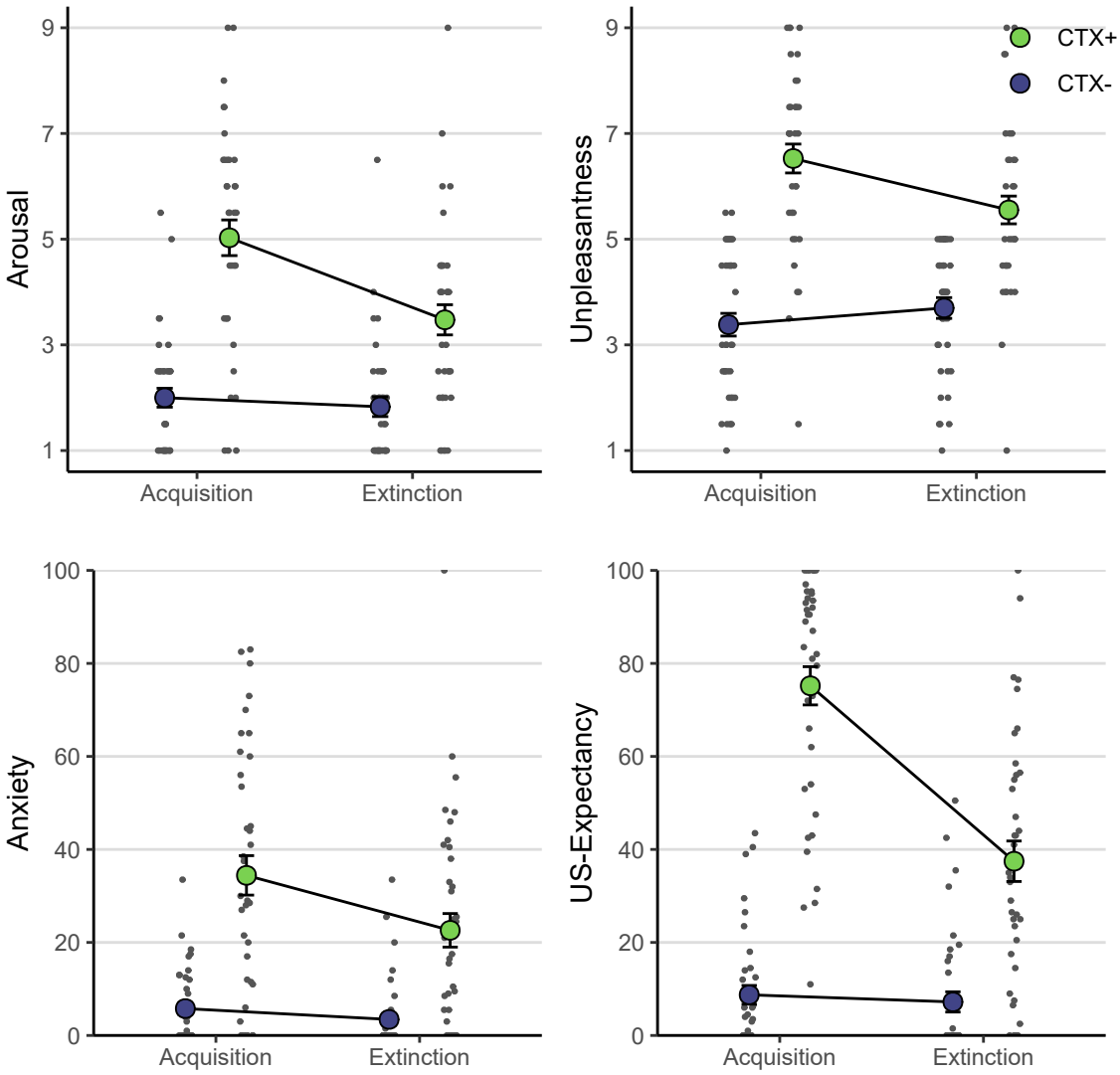

Figure 5.8: Mean arousal, unpleasantness, anxiety and US-expectancy ratings (±SEM) of the video stimuli.

5.4 Discussion

In the present study, a differential context conditioning paradigm was used to investigate visuocortical activity during potential threat and extinction learning. For the first time, steady-state visual evoked potentials were induced by presentations of flickering video stimuli. During each video, participants were passively guided on

a pre-recorded pathway through one of two virtual offices. To capture physiological and behavioral responding during potential threat, skin conductance responses were collected next to verbal reports of subjective valence, arousal, anxiety and US-expectancy.

Results demonstrated successful context conditioning for subjective measures of defensive responding. After acquisition, the anxiety context elicited higher arousal, unpleasantness, anxiety and US-expectancy ratings than the neutral context. These results were substantiated by a marginally significant effect of context conditioning on skin conductance responses to the onset of the video stimuli. Consequently, these findings contribute to a vast body of literature, demonstrating enhanced defensive responses during situations of potential threat (e.g. Grillon et al., 2004, 2006; Andreatta, Glotzbach-Schoon, et al., 2015; Glotzbach-Schoon, Tadda, et al., 2013).

Please note, that this study only found a marginally significant effect for skin conductance responses during fear acquisition and extinction. One possible explanation for this is that skin conductance responses were measured to the onset of the video stimuli. During these videos, however, participants started on a corridor in front of a closed door to the room and the door did not open until two seconds after video onset. Therefore, participants were not able to identify the upcoming condition during this initial interval, leading to a separation of the orientation response to the stimulus onset and the response to the motivational significance of the upcoming condition (Bradley et al., 2012; Sokolov, 1963). To take this into account, skin conductance responses were scored during an interval after the initial two seconds. However, this approach is only a crude way to quantify emotional modification of electrodermal activity and considerable noise remains due to interindividual variability among the time intervals needed to recognize the upcoming condition. This unsystematic variability may have led to a reduction of context conditioning effects. Moreover, the present study was not able to infer the timepoint of the video, at which

the participant became aware of the current condition. Other studies used skin conductance levels (SCLs) to measure electrodermal activity during context conditioning (e.g. Glotzbach-Schoon et al., 2015). In the present study, however, the presentation of electrical stimuli at random time points in combination with short ITIs results in systematic artifacts during the anxiety context. Therefore, future studies could use longer ITIs and more US free trials to avoid confounding effects of electrical stimulation on skin conductance levels.

To quantify sustained visual activity, steady-state visual evoked potentials were measured during an initial as well as a sustained time window. The initial time window comprised the first seven seconds after stimulus onset, though the first two seconds were omitted from statistical analysis, as participants were not aware of the condition at this timepoint due to closed doors. The sustained time window was a randomly chosen interval of five seconds during the second half of the video stimulus presentation, which was not further signaled to the participant.

Analyzing the signal-to-noise ratios of the 20 Hz driving frequency during the initial response window revealed no differences between the anxiety and the neutral context for acquisition or extinction learning. However, the steady-state responses evoked by the flickering video stimuli revealed generally high signal-to-noise ratios and descriptive topographical analysis showed broad visuocortical activity over the occipital scalp (see also Fig. 5.2), paralleling findings of other contextual threat ssVEP studies (Kastner et al., 2015; Kastner-Dorn et al., 2018; Wieser & Keil, 2014; Wieser et al., 2016b). These results suggest successful induction of ssVEP signals by flickering video stimuli, which seemed, however, not to be sensitive to potential threat, at least not during the initial response interval. Using flickering screenshots of the virtual offices, Kastner et al. (2015) found differential effects of context conditioning throughout the whole duration (20 s) of the stimulus presentation. However, fine-grained temporal analyses revealed that these effects were mainly driven by differ-

ences throughout the later time intervals of the context presentation (beginning from 8 s after context onset), suggesting that changes in visuocortical activity due to potential threat do not appear immediately but become operational throughout longer periods of potential threat, especially with contexts being more complex. Equally, in the present study, effects of context conditioning on ssVEP amplitudes might not have yet occurred during the initial response window. It is not surprising that potential threat is associated with effects on a larger time-scale, as danger is not yet imminent and there is no need for a rapid engagement of defensive mechanisms (Davis et al., 2010; Fanselow, 1994). This idea is supported by the results of the fMRI-study by Andreatta et al. (2015), demonstrating that during sustained context presentations different neural regions are activated than during the initial response window. Crucially, enhanced amygdala activity was only found during the sustained response window. Given its important role in driving changes in cortical sensory processing (Miskovic & Keil, 2012), the absence of amygdala activity could be accompanied by a lack of differential visuocortical responding during the initial presentation of the contexts.

During the sustained response window, the present study found differences in ssVEP amplitudes between the anxiety and neutral context. Against expectations, however, visuocortical activity during the anxiety context was decreased compared to the neutral context. This finding is in contrast to the results of Kastner et al. (2015), who found increased activity during the anxiety context. The most likely explanation for this finding is that the decreased visuocortical activity was a result of artifacts by the electrical stimulation during the anxiety context. Even though no USs were presented during the sustained response interval, the minimum temporal distance between an electrical stimulus and the sustained response window was only two seconds. While electrical stimuli usually only have short-term effects on the EEG signal, they might have affected ongoing ssVEP responses, leading to a disruption of the neural

entrainment and synchronicity. As a consequence, time-locked power of the ssVEP signal would be reduced. To take this into account, I re-ran the ssVEP analysis by excluding all trials with electrical stimuli during an interval of 5 seconds or less prior to the sustained response window. Crucially, the anxiety context remained associated with decreased visuocortical activity compared to the neutral context. A more in-depth analysis of the visuocortical activity as a function of the total number of preceding aversive events during the sustained response interval of the anxiety context demonstrated that even anxiety context trials, which were not associated with aversive events prior to the sustained response interval, elicited smaller ssVEP amplitudes than the neutral context. It is also possible that participants felt relieved after already having experienced aversive events prior to the sustained response interval. It is well known that pain relief learning leads to attenuated defensive responses (Andreatta et al., 2012, 2010). However, result patterns of the present study yielded no evidence for relief-attenuated visuocortical responses, as the number of preceding US was not associated with decreased ssVEP amplitudes during the anxiety context.

Another explanation for reduced ssVEP responses during the anxiety context is that the induction of potential threat has led to more eye-movements than the neutral context. Richards et al. (2014) suggested that hypervigilance induced by potential threat is associated with excessive scanning of the environment, which can be achieved by increasing eye-movements. Importantly, eye-movements do not only cause artifacts on the EEG signal themselves, for example via muscle activity during moving the eyeball (Croft & Barry, 2000), but small saccades can also lead to a disruption of the neural entrainment by the flickering stimulus and consequently reduce ssVEP amplitudes. To test the hypothesis that the anxiety context was associated with more eye-movements than the neutral context, the present study utilized the EOG electrodes of the EEG system to quantify eye-movement activity during the sustained response interval. Subsequent analysis revealed no differences between the

anxiety and the neutral context. Yet, it is important to mention that quantifying eye-movement via EOG signals results in a rough measure of eye-movement activity only and should be complemented by more sensitive methods of eye-tracking in future studies.

Notably, the present findings parallel results of a recent study (Campagnoli et al., 2019), utilizing ssVEPs to investigate visuocortical responses to subtle changes of emotional facial expression. The authors presented flickering pictures of neutral facial expressions, which throughout the trial changed to either another neutral or to an emotional facial expression of the same individual. Crucially, it was observed that transient changes of the facial expression perturbed the ssVEP signal and lead to a reduction of the time-varying ssVEP amplitudes. Similar disruptions of the ssVEP signal by transient changes have been observed before and could be demonstrated using pictures of neutral and affective scenes (Bekhtereva et al., 2018). It has been suggested that the phase of the neural oscillation is disrupted by transient brain responses, as underlying circuits receive additional afferent input, interfering with the ssVEP phase and ultimately resulting in a reduction of the ssVEP power (Campagnoli et al., 2019; Moratti, Clementz, Gao, Ortiz, & Keil, 2007; Muller, Andersen, & Keil, 2008). Applied to the present paradigm, video stimuli can be considered as a continuous stream of afferent input, as each frame of the video stimulus contains additional sensory information. This is an important aspect, since the neural mechanisms underlying perceptual processing of continuously changing visual stimuli are not well understood. While studies using static images to induce ssVEPs usually target specific low-level features of the visual stimulus, like orientation, contrast or color (Keil et al., 2013; McTeague et al., 2015) or employ pictures of static facial expressions to investigate social attentional processing (e.g. Wieser et al., 2014a, 2011; Stegmann, Ahrens, Pauli, Keil, & Wieser, 2020), the video stimuli of the present study consist of a complex composition of different low-level features that, in addi-

tion, change over time. To improve our understanding of visuocortical processing of video stimuli, future studies should systematically investigate visuocortical responding to visual stimuli that continuously change in one low-level feature, for example rotating grating-stimuli that continuously change in orientation.

Even more strikingly, studies investigating visuocortical responding during transient changes of visual stimuli found consistent evidence for an enhanced perturbation of the ssVEP signal by emotional or motivationally relevant compared to neutral stimuli (Bekhtereva & Müller, 2017; Bekhtereva et al., 2018; Campagnoli et al., 2019). This finding can be transferred to the results of the present study, which revealed decreased ssVEP amplitudes during the sustained response interval for the anxiety compared to the neutral context, suggesting a greater disruption of the ssVEP signal during potential threat. It remains unclear, however, which mechanisms are responsible for the disruption of the ssVEP signal by changing stimuli and how these mechanisms are modulated as a function of emotional and motivational relevance of those stimuli.

It is also important to mention that effects of context conditioning were relatively stable throughout extinction learning for measures of defensive responding, while visuocortical responses showed no further modulations by the anxiety context. Until now, no study has directly compared the temporal dynamics of visuocortical and physiological responses during extinction learning. However, recent studies analyzing ssVEPs on single-trial level demonstrated a reduction of threat-enhanced ssVEP amplitudes after as few as two unreinforced CS+ presentations (McTeague et al., 2015; Wieser et al., 2014b). This finding is also in line with the different functions of threat detection and defensive responding. Taking an evolutionary perspective, it is crucial for an organism to adapt sensory processing to changing environments, especially, if threat-signaling stimuli stop predicting aversive events. Consequently, the rapid extinction of threat-enhanced visuocortical responses could represent the

ability to quickly reorient sensory processing, while the 'efferent' readiness to respond to threat remains, because false alarms are less costly than a - potentially lethal - miss (Miskovic & Keil, 2012; Stegmann et al., 2020). However, further research is necessary to disentangle the temporal dynamics of sensory processing and defensive responding during extinction learning.

In conclusion, situations of potential threat prompts activation of the defensive system, which is associated with facilitated defensive responses on a subjective and physiological level (Davis et al., 2010; Evelyn Glotzbach et al., 2012; Grillon et al., 2004). Findings regarding ssVEP amplitudes demonstrated generally high signal-to-noise ratios, while differential responding was only evident during sustained response intervals. Therein, enhanced disruption of the ssVEP signal suggests interferences by a continuously changing video stream which is enhanced as a function of motivational relevance (Campagnoli et al., 2019). These findings contribute to our understanding of the perceptional processing of more ecologically valid context stimuli and its modulation during situations of potential threat.

General Discussion

The main goal of this thesis is to elucidate defensive responding as well as the neural correlates of attentional processes during fear, anxiety and their combination. As noted in the neuroscience literature, fear is an emotional state in response to acute, imminent threat, while anxiety describes a prolonged state of apprehension and hypervigilance during situations of diffuse, potential threat (Davis et al., 2010; Sylvers et al., 2011). Accordingly, the present thesis utilized different paradigms to establish acute and potential threat in order to systematically induce fear and anxiety, respectively. Fear and anxiety are closely related and both serve the purpose of protecting the organism from harm. To achieve this end, they rely on similar defensive mechanisms and both mobilize defensive resources to cope with the threatening situation, although to a different degree. However, there are also important differences between fear and anxiety. The most prominent distinction involves the function of attentional processes during these states. It has been suggested that fear prompts selective attention, while anxiety is associated with heightened vigilance in general. Yet, a direct neurophysiological account for selective attention and hypervigilance during acute and potential threat remains obscured. At the same time, even though they are probably distinct emotional states, fear and anxiety are not necessarily mutually exclusive. First conceptual frameworks and theories focusing on the neural networks raised the hypothesis of potential interactions between fear and anxiety (Fox & Shackman, 2019).

Accordingly, the present thesis tested three specific hypotheses regarding the interaction of fear and anxiety and the function of attention as well as defensive responses

therein. First, to elucidate neural correlates of selective attention and hypervigilance during acute and potential threat, Study 1 utilized steady-state visual evoked potentials during a modified version of the NPU-threat task and compared high with low anxious individuals to investigate the functional relevance of these mechanisms for different levels of trait-anxiety.

Second, Study 2 and 3 aimed at investigating potential interactions between fear and anxiety on the visuocortical, behavioral and physiological level. To this end, steady-state visual evoked potentials were measured next to skin conductance responses as well as behavioral ratings during a novel, orthogonal combination of a cue and context conditioning paradigm. The main hypothesis was tested that acute threat cues prompt stronger defensive and visuocortical responses during contexts of sustained anxiety compared to neutral contexts.

Third, to elucidate the neural correlates of hypervigilance on a visuocortical level and to investigate these processes during a more ecologically valid context conditioning paradigm, Study 4 quantified steady-state visual evoked potentials during presentations of flickering video stimuli.

6.1 Visuocortical correlates of hypervigilance and selective attention

As the common hypothesis underlying all of this thesis' studies, I expected the neural correlate of selective attention to be associated with potentiated ssVEP responses to the acute threat-signaling stimuli, while hypervigilance should be characterized by heightened visuocortical responding to the visual stimuli, which represent the potentially threatening context condition.

This hypothesis was explicitly tested in Study 1, which investigated ssVEPs during a modified version of the NPU-threat task (see also Wieser et al., 2016b), to

disentangle visuocortical responses to acute threat-associated central cues or potential threat-associated contexts. For low-trait-anxious individuals, results revealed heightened visuocortical responses to the central cue in the predictable threat condition, which indicated acute threat. At the same time, ssVEP responses to the contexts yielded higher amplitudes during the unpredictable and predictable threat conditions compared to the neutral condition, suggesting that contexts, which signal potential danger, prompt enhanced visuocortical processing. Taken together, these results demonstrate further evidence for neural correlates of selective attention to acute threat and hypervigilance during potential threat. These findings are in line with the results of two recent studies investigating visuocortical processing during predictable and unpredictable threat. Using slightly altered methods (e.g. different timing and the same central stimulus for all conditions), Wieser et al. (2016b) also found enhanced visuocortical responses to the central cue in the predictable threat condition and to the contexts in the unpredictable condition. In addition, the authors could show that contexts during the presentation of the central cue in the predictable threat condition were enhanced as well, which seems to be inconsistent with the notion of selective attention. It is important to mention, however, that in their study, the central cue had only a predictive value in combination with the contexts (since the same central cue was used for all conditions), which could have led to a saliency gain for the contexts in the predictable threat condition. Importantly, in Study 1 of the present thesis, where different central stimuli were used per condition, this result could not be replicated. Secondly, Kastner-Dorn et al. (2018) used screenshots of virtual offices as contexts and different virtual avatars as central cues to investigate ssVEPs during a NPU-threat task. Patterns of visuocortical responding mirrored closely the results of the present study, evident in enhanced ssVEP amplitudes to the central cue in the predictable threat condition and enhanced amplitudes to the contexts in the unpredictable and predictable threat conditions compared to the neutral

condition.

In summary, results of Study 1 along with findings from the recent literature demonstrate further evidence for neural correlates of selective attention and hypervigilance to acute or potential threat, respectively. Crucially, these findings match theoretical accounts for the function of attentional processes during acute and potential threat (Richards et al., 2014). In this framework, heightened visuocortical responses to discrete stimuli that signal acute threat can be interpreted as a neural correlate of selective attention. During selective attention, the attentional focus is selectively narrowed on the threatening stimulus, which is preferentially processed in order to closely monitor the danger and to prepare for upcoming defensive responses (Lang et al., 2000). Conversely, hypervigilance is accompanied by a broadening of attention during situations of potential threat, which serves to monitor the surroundings for upcoming danger and facilitate threat detection. In line, enhanced visuocortical processing of contexts might represent an electrocortical marker of hypervigilance.

Furthermore, findings of Study 1 indicate that visuocortical processes seem to be dysfunctional in high anxious individuals, as they did not discriminate between the different context conditions and showed diminished visuocortical responses to the contexts signaling predictable and unpredictable threat compared to low anxious individuals. These results in combination with the generally elevated subjective threat responses for high anxious individuals could be interpreted in the context of aberrational processing of potential threat in pathological anxiety (Gorka et al., 2017b; Gorka, Nelson, & Shankman, 2013; Grillon et al., 2008, 2009). Measuring anxiety-potentiated startle responses, these studies demonstrated consistent evidence for enhanced startle responses to potential, but not acute threat in patients with panic disorders, PTSD, specific and social phobia compared to healthy controls. However, further studies need to investigate if an impaired ability to perceptually discriminate between threat and neutral context conditions is causally related to enhanced

responding on physiological measures of defensive responses.

In this regard, Study 2 and Study 3 investigated interactions between responding to actual and potential threat. However, they could find evidence for neural correlates of neither hypervigilance during potential threat nor selective attention to acute threat. Study 3 could only show weak evidence for enhanced ssVEP responses to the actual threat stimulus compared to the neutral stimulus in an a priori acquisition phase. Compared to Study 1, it is important to mention that Study 2 and 3 tried to simultaneously analyze mechanisms of hypervigilance and selective attention. Using the NPU-threat task in Study 1, potential and acute threat - and consequently mechanisms of hypervigilance and selective attention - were strictly separated by conditions. In Study 2 and 3, however, the orthogonal implementation of cue and context conditioning required the participants to monitor central and context cues simultaneously, which could have led to interferences on a visuocortical level. However, visuocortical competition analysis in Study 3 revealed no trade-off effects between central cue and context processing. Yet, ssVEPs have been analyzed during two concurrent tasks before and interferences between those tasks were seldomly found. For example, Keil et al. (2005) could show that electrocortical facilitation effects of emotional content of a stimuli and a visual search task combined additively. Equally, Boylan et al. (2019) found no visuocortical interferences between aversive learning and a concurrent feature selection task. Wieser et al. (2014) presented neutral versus emotional facial stimuli in front of neutral versus arousing background pictures. While the authors found the expected generally increased ssVEP amplitudes to arousing compared to neutral background pictures, there were no differences among visuocortical responses to the facial stimuli, suggesting interferences between visuocortical processing of facial stimuli and concurrent background stimuli. To my knowledge, Study 2 and Study 3 were the first studies to investigate visuocortical responses during two concurrent aversive learning tasks. Consequently, systematic investigations on how one threat-

related task affects the other on a visuocortical level are missing. First tendencies can be derived from a recent study, which systematically examined the effect of startle probes on a cue conditioning paradigm (Sjouwerman et al., 2016). The authors found that the application of startle probes interfered with cue conditioning and reduced differential defensive responding compared to a group, which went through the same cue conditioning paradigm without startle probes. Importantly, since startle probes are usually administered unpredictably and are perceived as highly unpleasant and aversive, they can be regarded as a means to induce potential threat. Consequently, this study showed first evidence that potential threat interfered with concurrent fear learning. This is why in Study 3, fear acquisition was conducted prior to the concurrent cue conditioning and threat-of-shock paradigm. Yet, similar interferences might be possible for visuocortical responses.

A reason for these interferences might be found on a neural level. Recent studies suggested that facilitation of sensory processes during fear acquisition are driven by synchronization of neural oscillations between distributed neural areas (Miskovic & Keil, 2012). Crucially, steady-state visual evoked potentials also represent strongly phase-aligned neural oscillations (Moratti et al., 2007). Furthermore, interfering with the theta-band coupling between amygdala and hippocampus in rodents reduced the recall of conditioned fear (Lesting et al., 2011). Therefore, phase-alignment and synchronization of neural oscillations are assumed to play an important role in the neural mechanisms of aversive learning as well as in the generation of ssVEPs. It is not well understood, however, how the implementation of two aversive learning processes interact with the generation of ssVEPs in response to stimuli flickering in different frequencies. It might be possible that the phase-alignment during fear conditioning interferes with the synchronization during the generation of the ssVEPs, resulting in a disruption of the ssVEP signals, in particular, if there are two or more concurrent aversive learning tasks. More systematic research is needed on how visuocortical

processing of two conditioned stimuli is affected by interfering with underlying neural oscillation processes. It is also important to note, that these findings are primarily based on cue conditioning paradigms and that it has not been examined yet, if the same or similar processes apply for sensory mechanisms during situations of potential threat.

Investigating visuocortical processes during situations of potential threat was the main goal of Study 4. To induce ecologically more valid representations of contexts, Study 4 used flickering video stimuli, in which participants were guided through virtual offices. Potential threat was induced by the administration of unpredictable electrical stimuli during one, but never during another office. Contrary to expectations, results revealed decreased ssVEP responses during the anxiety compared to the neutral context. However, this result is most likely related to the presentation of flickering video stimuli and not a genuine visuocortical response to potential threat. For example, by using flickering screenshots of similar virtual offices, two recent studies demonstrated enhanced ssVEP amplitudes in response to potential threat throughout the whole duration of the context presentation (Kastner et al., 2015; Kastner-Dorn et al., 2018). Equally, replicating findings of Wieser et al. (2016b), Study 1 revealed enhanced visuocortical activity during threatening contexts, using peripherally presented geometrical symbols as contexts. However, a neural account for ssVEPs in response to video stimuli remains elusive. Recently, studies started to investigate how transient changes of the presented visual stimuli affect the ssVEP response. To this end, Bekhtereva et al. (2017; 2018) used a rapid serial visual presentation (RSVP) stream, which included pictures of neutral und unpleasant scenes. To induce ssVEPs, the RSVP stream was presented in different frequencies ranging from 3 to 8.75 Hz. As expected, transient changes from neutral to unpleasant stimuli were associated with enhanced ssVEP amplitudes for the 3 Hz, 4 Hz, and 8.57 Hz frequency. Using a flicker frequency of 6.66 Hz, however, revealed the exact opposite, i.e. changes from

neutral to unpleasant stimuli actually decreased ssVEP response amplitudes. This finding is specific for the 6.66 Hz frequency and could already be replicated in two different experiments (Riels, Rocha, & Keil, 2020). By using simulation analysis, Bekhtereva et al. (2017) demonstrated that this effect might be caused by a linear superposition of the ERPs, which are evoked by the individual images of the RSVP, that may lead to a disruption of the ssVEP pattern and ultimately reduce ssVEP amplitudes. If linear superpositions of ERPs resulted in decreased ssVEP amplitudes, similar mechanisms ought to be expected with presentation frequencies of integer multiples of the 6.66 Hz frequency. Crucially, Study 4 used a presentation frequency of 20 Hz, which is exactly three times 6.66 Hz, suggesting that linear superposition effects could have ocured. Consequently, future studies should replicate the results of Study 4, using a slightly different presentation frequency and then expecting enhanced ssVEP amplitudes during the anxiety context. Another study investigating visuocortical responses to transient changes of facial stimuli revealed a disruption of the ssVEP signal by the transient changes in general (Campagnoli et al., 2019). Crucially, this disruption was enhanced for changes from neutral to angry compared to neutral to neutral facial expressions. Taken together, there is growing evidence that visuocortical activity during changes of the visual stimuli is influenced by methodological as well as affective features of the stimuli. Studies investigating the interplay of these features could offer promising new insights into sensory mechanisms during potential threat and during more ecologically valid stimuli in general.

6.2 Defensive responding during fear, anxiety, and their interaction

The second major goal of this thesis was to examine behavioral and psychophysiological indices of defensive responding during acute threat, potential threat, and their

combination. Defensive responding in relation to the physical or temporal distance to a threat has thoroughly been investigated in the context of the threat-imminence model (Blanchard & Blanchard, 1989; Fanselow, 2018; Fanselow & Lester, 1988; Lang et al., 1997, 2000), which divides defensive mechanisms into the pre-encounter, post-encounter, and circa-strike stage. During the pre-encounter stage, potential threat is expected, but has not yet been identified, leading to hypervigilance, a feeling of anxiety and worry and an increase in autonomic arousal. Once the threat has been detected (post-encounter stage), attention is selectively focused on the source of the danger, heart rate decelerates, startle-reflexes are potentiated and autonomic arousal further increases, which is usually associated with a feeling of fear (Hamm, 2020). The circa-strike stage encompasses the events around the inevitable contact with the threatening stimulus, eliciting the actual fight-or-flight response. In the laboratory, induction of potential threat models aspects of the pre-encounter stage, while acute threat can be used to model the increased imminence during post-encounter. Importantly, the threat-imminence model predicts an increase in autonomic arousal and a potentiation of defensive responses from pre- to post-encounter, which is associated with a transition from a feeling of anxiety to fear. It is important to note, however, that the threat-imminence model depicts anxiety and fear on a single continuum, where either one of both states is active at a time. Accordingly, the threat-imminence model suggests that threat-related features of the context (potential threat/anxiety versus neutral context) do not influence the magnitude of defensive responses to acute threats (fear responses). On the other hand, there is growing evidence that considerable interplay between fear and anxiety exists (Fox & Shackman, 2019; Shackman & Fox, 2016), which is evident on a neural level in humans (Andreatta, Glotzbach-Schoon, et al., 2015; Somerville et al., 2013, 2010), and for animal models (Davis et al., 2010; Ponder et al., 2007). These findings suggest that fear responses are potentiated if the threatening stimulus is presented during an anxiety compared to a neutral

context.

To test these alternative hypotheses, Study 2 and 3 orthogonally combined cue and context conditioning, so that the magnitude of defensive responses to acute threat (CS+) can be compared between anxiety (CTX+) versus neutral (CTX-) contexts. The crucial tests for skin conductance responses and subjective indices of defensive responding revealed enhanced responses to the acute threat-signaling stimulus during the anxiety compared to the neutral context. However, this effect was not specific for the CS+, but could also be found for the CS-, suggesting that anxiety induced by potential threat does not specifically potentiate fear responses, but generally facilitates defensive behavior, which combines additively with the magnitude of defensive responses to the fear stimulus. These findings are in line with the notion that there is a functional interplay between fear and anxiety, and consequently disagree with the idea that both states are mutually exclusive (see also Davis et al., 2010; Fox & Shackman, 2019; Tovote et al., 2015). Yet, it is important to mention that these findings are still consistent with predictions made by the threat-imminence model, which states that the magnitude of defensive responses increases from pre- (potential threat) to post-encounter (acute threat). At the same time, the threat-imminence model does not consider encounters with acute threat outside of potentially threatening situations. From this perspective, findings of the present studies indicate that fear responses during neutral situations are weaker compared to fear responses during potentially threatening situations. Bearing in mind that the magnitude of fear responses is directly related to the organism's chances of survival (Blanchard & Blanchard, 1989; Mobbs et al., 2015), these findings emphasize the functional role of anxiety for upcoming fear responses. Furthermore, these results suggest that defensive responding is organized in an additive, multi-staged manner, which fits well with the main idea of the threat-imminence model. Entering potentially threatening situations (pre-encounter stage) activates a low amount of defensive resources to prepare

for upcoming danger. Once an acute threat has been detected (post-encounter stage) and physical contact has gotten more likely, further resources are mobilized in order to facilitate defensive responding.

Future studies need to investigate the underlying mechanisms and reasons of why encountering the same acute threat within neutral situations (compared to potentially threatening situations) results in a in total reduced mobilization of defensive resources. Two explanations should be considered: On one hand, hierarchically responding to threat according to its imminence increases the efficiency of the organism's defensive behavior (Mobbs et al., 2015). On the other hand, timing and response latencies might play an important role. Resources activated in response to acute threat might be quickly accessible but short-lasting, while resources activated in response to potential threat could be more sustainable but need more time to mobilize. Organizing defensive resources in this multi-staged manner would satisfy the demands posed by the environment in the context of the threat-imminence model and suggests that two separate pools of resources could have developed through evolutionary history. The ability to tap resources from two separate pools could also explain the additive effects of fear and anxiety on measures of defensive responding. Further evidence for this notion stems from studies investigating the neural correlates of fear and anxiety, revealing short-lived central amygdala outputs during acute threat and more slowly recruited BNST outputs during potential threat (Herrmann et al., 2016; Perusini & Fanselow, 2015; Somerville et al., 2013). However, investigating neural responses during orthogonally combined cue and context conditioning constitutes an important next step to further deepen our understanding of the interplay between fear and anxiety.

6.3 Limitations

All of this thesis' studies utilized ssVEPs to quantify visuocortical activity during acute and potential threat. SsVEPs provide a powerful tool for investigating sensory processes. Its high signal-to-noise ratio (Norcia et al., 2015), even on the level of single trials (Keil et al., 2008; Wieser et al., 2014b), as well as its reliability (Wieser et al., 2016a) are well established. It provides a means to quantify sustained sensory processing (Muller et al., 1998) and is able to index the dynamics therein due its fine temporal resolution (Bekhtereva et al., 2018; Campagnoli et al., 2019; Kastner et al., 2015). Furthermore, using frequency tagging, ssVEPs allow to quantify visuocortical responses to two or more visual stimuli presented at the same time (Wieser & Keil, 2014; Wieser et al., 2011). However, the use of ssVEPs comes with certain limitations that need to be considered. The visual entrainment of the neural oscillations interferes with the analyses of other frequency bands. For example, differential fear conditioning was evident in higher frontomedial theta (4-8 Hz) power (Sperl et al., 2019) and a suppression of visuocortical alpha (8-12 Hz) (Panitz, Keil, & Mueller, 2019) in response to the CS+ compared to the CS-. Importantly, the analysis of phase-locked components of the ssVEP via averaging in the time-domain prior to spectral analysis obscures the presence of any non-phase-locked neural oscillations. At the same time, the entrainment of the ssVEP is known to result in a phase alignment of neural background oscillations and therefore might distort natural oscillations near the driving frequency (Moratti et al., 2007). Even though ssVEPs are well suited to index visuocortical processes, they have limited use for investigating processes at other brain regions. Like other EEG measures, ssVEPs have a limited spatial resolution and should be used with caution for drawing conclusions about deep brain structures, like the hippocampus or amygdala, which play a key role in the neural networks underlying fear and anxiety (Davis et al., 2010; Tovote et al., 2015). To

overcome this gap, future studies could combine the high spatial specificity of fMRI with the excellent temporal resolution of EEG measures.

The occurrence of aversive events (electrical stimuli and noise blasts) during the anxiety contexts to induce potential threat are well known to result in strong artefacts on the EEG signal. As a consequence, the use of high reinforcement-rates (75-100% per trial) in this study constrains the analysis of the whole context presentation. Accordingly, present ssVEP analysis focused on context onsets, contexts during central cue presentations and implementations of US-free intervals in contrast to whole trial analyses. However, whole trial analyses are crucial to investigate changes in continuous visuocortical responding. In particular for Study 4, continuous analyses could reveal further insight into the suppression of visuocortical activity during the anxiety compared to the neutral context.

To index physiological components of the defense response, the present studies recorded electrodermal activity only. Transient skin conductance responses are a well-established measure to demonstrate differential responses to acute threat (Bach et al., 2010; Lonsdorf et al., 2017) and tonic skin conductance levels have been proven to be effective in differentiating between neutral contexts and contexts of sustained anxiety (Glotzbach-Schoon et al., 2015; Glotzbach-Schoon, Andreatta, et al., 2013). Due to high rates of aversive events, analyses of skin conductance levels would have been confounded with electrodermal responses to the US at the offset of the CS+ and by the unpredictable US in the anxiety context. For this reason, the present studies focused on the analysis of skin conductance responses to visual stimuli onsets. However, US-related SCRs still interfered with SCRs to the visual stimuli onset, particularly in the acquisition phase of Study 2, where unpredictable aversive events might have reduced SCR amplitudes to the central stimuli in the anxiety context. Moreover, SCRs had limited use for the analysis of the video stimuli onsets in Study 4, as the stimulus onset did not align with the recognition of the trial's condition.

During the video stimuli, participants were placed in front of a closed door, which only opened after the first second. To overcome these issues, future studies should use reduced reinforcement rates and focus on the analysis of US-free trials for electrodermal activity only. Another possibility might be to analyze electrodermal activity via more complex deconvolution algorithms, which are capable of disentangling tonic and transient components of the skin conductance responses (Bach et al., 2010; Ojala & Bach, 2020).

It is also crucial to seek convergent evidence from different measures of defensive responding, like cardiovascular activity or modulations of the startle reflex. For example, studies comparing responses to acute and potential threat usually measure probed startle reflexes during the NPU-threat task (Grillon et al., 2006, 2008, 2009). Startle reflexes are elicited by presenting short, loud noise blasts. In contrast to electrodermal activity, startle responses only provide a point measure of the organism's affective state, however, multiple startle probes can be administered during sustained context presentation, to obtain an index of ongoing affective information processing (Grillon, 2002). It is important to mention, however, that startle responses should not be used heedlessly for investigating interactions of acute and potential threat. As mentioned above, startle probes are loud noise blasts, which are usually perceived as unpleasant. Consequently, unpredictable administration of startle probes has the capacity to induce additional potential threat, which might interfere with the experimental manipulation of threat, e.g. context conditioning.

Study 2 and 3 used orthogonal combinations of cue and context conditioning to investigate defensive responding to the fear cue during an anxiety compared to a neutral context. Please note, however, that the neutral context was not completely neutral. Because of the orthogonal design, fear cues, and consequently aversive events, were presented during the anxiety context as well as during the neutral context. Even though aversive events are primarily linked to the fear cue, they can become associated

with the context, in which the events occur (Baas et al., 2008). Using computational models, Yuan et al. (2018) inferred that differential cue conditioning also increased the associative strength between the context and an aversive event. Accordingly, by presenting cue-related aversive events in the neutral context, it becomes associated with these events, although to a lesser extent than the central cue. As a consequence, the neutral context gains motivational relevance over time. This mechanism could also explain the enhanced visuocortical responses to the contexts during the P-condition in Study 1 and in the study by Kastner-Dorn et al. (2018). In Study 2 and 3, however, it could have led to diminished differential responses between the anxiety and the neutral context, as the neutral context becomes associated with cue-related aversive events and thereby gains predictive value for potential threat as well. It is important to mention, however, that the anxiety context probably elicited more intense anxiety than the neutral context, as it was associated with additional means of inducing potential threat (unpredictable electrical stimuli in Study 2 and verbally instructed expectations of electrical stimuli in Study 3). Crucially, computational models suggest that indirect CTX-US associations are built more slowly than direct CS-US associations (Yuan et al., 2018). Therefore, future studies should analyze these temporal dynamics to compare the impact of indirect CTX-US associations on the interaction of fear and anxiety between the beginning and the end of the orthogonal test phase.

6.4 Summary and outlook

The major goals of this thesis were to 1) provide a neural account of hypervigilance and selective attention during potential and acute threat and to 2) investigate interactions between fear and anxiety. In four studies, different combinations of aversive cue and context conditioning have been realized as laboratory models of acute and

potential threat. To quantify visuocortical correlates of attentional mechanisms, all studies measured steady-state visual evoked potentials by the means of EEG. In line with the recent literature (Kastner-Dorn et al., 2018; Wieser et al., 2016b), present results suggest further evidence for the neural correlates of selective attention and hypervigilance, reflected in enhanced visuocortical responses to visual stimuli signaling acute or potential threat, respectively. Findings for concurrent implementations of acute and potential threat and for more complex video stimuli are less consistent, however. While ssVEP amplitudes were not sensitive to orthogonal combinations of acute and potential threat, they even demonstrated reduced visuocortical activity during flickering video stimuli associated with potential threat compared to neutral video stimuli. In conclusion, these findings indicate that ssVEPs can be used to index visuocortical correlates of attention during fear and anxiety, but more research is necessary on how the visual brain responds to threatening stimuli in more complex and ecologically valid paradigms.

Regarding measures of defensive responding, the present thesis found first evidence for additive interactions between fear and anxiety. While recent models suggest that fear and anxiety are mutually exclusive or lie on a single continuum related to the organism's temporal or physical distance to a threat (see Fig. 6.1B) (Blanchard & Blanchard, 1989; Fanselow, 2018; Fanselow & Lester, 1988; Lang et al., 1997, 2000), present results for behavioral and physiological indices of defensive responding demonstrated potentiated fear responses during anxiety compared to neutral contexts. These findings suggest an additive, multi-staged organization of defensive responses to threatening stimuli and highlight the functional relevance of anxiety and fear in facilitating survival behavior (see Fig. 6.1C). In this model, entering the pre-encounter stage triggers anxiety, mobilizing a low amount of defensive resources to prepare for potential threats. On the post-encounter stage, after encountering threat becomes more likely, fear arises in addition to anxiety in order to mobilize further defensive

resources. An additive model of fear and anxiety offers promising new perspectives for

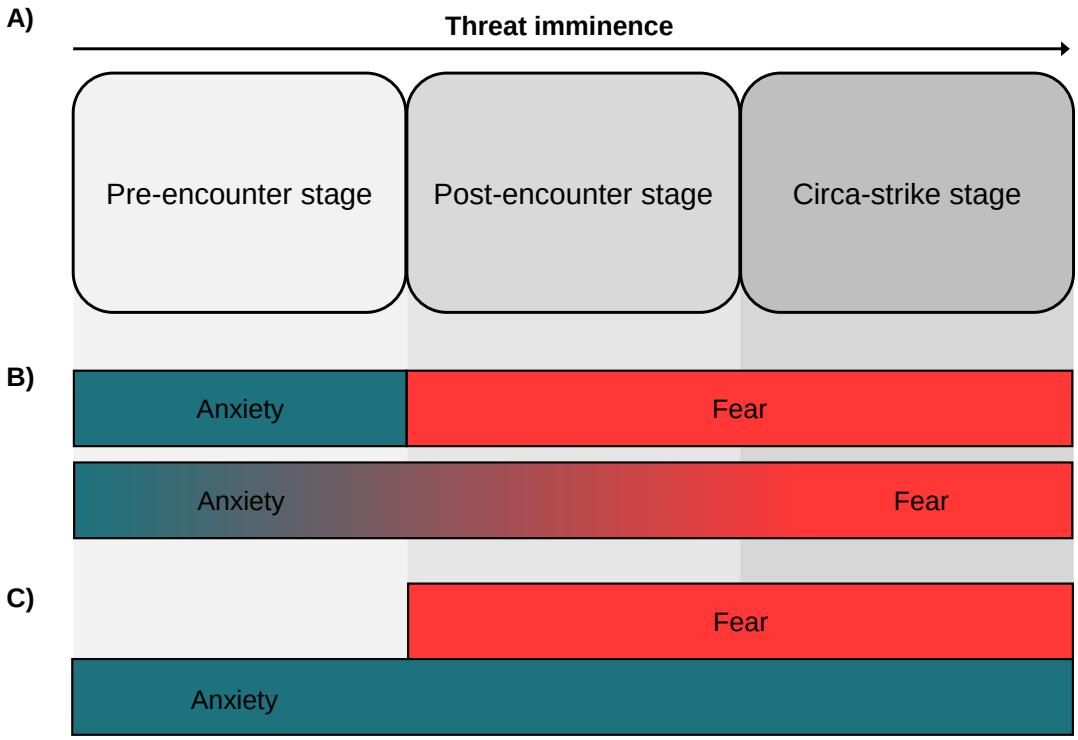

Figure 6.1: An updated model of how fear and anxiety map onto the threat-imminence-continuum. A) The three stages of the threat-imminence model. B) Illustrations of recent models, in which fear and anxiety are mutually exclusive (upper row) or lie on a single continuum instead of being discrete emotions (lower row). C) Depiction of an additive model between fear and anxiety, where anxiety is associated with the pre-encounter stage, while the post-encounter triggers additional fear responses.

fundamental and clinical research alike. It is well in line with recent notions of Fox et al. (2019) regarding the neural networks underlying fear and anxiety, demonstrating extensive overlap and co-activation of the amygdala and the BNST in response to a wide range of threats instead of being strictly separated networks. However, even though the circuits of fear and anxiety are well characterized (Davis et al., 2010; Tovote et al., 2015), their interaction on a neural level remain elusive. Advanced tools, like in vivo imaging and optogenetics, can be used in animal models to test hypotheses derived from the additive model regarding the neural underpinnings of

fear and anxiety. Equally, future studies could use fMRI methods to elucidate the interplay between the neural networks of fear and anxiety in humans.

An interaction between fear and anxiety could also provide a potential candidate for a novel biomarker of clinical anxiety. Within the RDoC framework, potential threat (anxiety) and acute threat (fear) are separated subsections of the negative valence systems (Cuthbert, 2014). Anxiety disorders have been suggested to be characterized by aberrational processing of potential threat (Gorka et al., 2017b; Grillon et al., 2008, 2009). At the same time, dysfunctional interactions between acute and potential threat processing might result in excessive defensive responses and disproportionate feelings of fear and anxiety. Hence, clinical anxiety is not necessarily related to the single subsections -acute or potential threat- but might rather be associated with a dysfunctioning regarding their interaction. Consistent with the findings above, dysfunctional neural circuits might exceed the normal, adaptive additive mechanism between fear and anxiety, ultimately leading to exaggerated physiological and subjective fear and anxiety responses (see also Levy & Schiller, 2021). Consequently, future studies could compare individuals with diagnosed anxiety disorders with healthy individuals to test dysfunctional interactions as a biomarker for pathological forms of fear and anxiety.

References

Ahrens, L. M., Muhlberger, A., Pauli, P., & Wieser, M. J. (2015). Impaired visuocortical discrimination learning of socially conditioned stimuli in social anxiety. *Soc Cogn Affect Neurosci*, *10*(7), 929–37. Journal Article. http://doi.org/10.1093/scan/nsu140

Alvarez, R. P., Chen, G., Bodurka, J., Kaplan, R., & Grillon, C. (2011). Phasic and sustained fear in humans elicits distinct patterns of brain activity. *Neuroimage*, *55*(1), 389–400. Journal Article. http://doi.org/10.1016/j.neuroimage.2010.11.057

Amaral, D. G., Behniea, H., & Kelly, J. L. (2003). Topographic organization of projections from the amygdala to the visual cortex in the macaque monkey. *Neuroscience*, *118*(4), 1099–1120.

American Psychiatric Association. (2013). *Diagnostic and statistical manual of mental disorders (dsm-5)* (5th ed.). Book, American Psychiatric Pub.

Andreatta, M., Fendt, M., Muhlberger, A., Wieser, M. J., Imobersteg, S., Yarali, A., … Pauli, P. (2012). Onset and offset of aversive events establish distinct memories requiring fear and reward networks. *Learn Mem*, *19*(11), 518–26. Journal Article. http://doi.org/10.1101/lm.026864.112

Andreatta, M., Glotzbach-Schoon, E., Muhlberger, A., Schulz, S. M., Wiemer, J., & Pauli, P. (2015). Initial and sustained brain responses to contextual conditioned anxiety in humans. *Cortex*, *63*, 352–63. Journal Article. http://doi.org/10.1016/j.cortex.2014.09.014

Andreatta, M., Leombruni, E., Glotzbach-Schoon, E., Pauli, P., & Muhlberger, A. (2015). Generalization of contextual fear in humans. *Behav Ther*, *46*(5), 583–96. Journal Article. http://doi.org/10.1016/j.beth.2014.12.008

Andreatta, M., Muhlberger, A., Yarali, A., Gerber, B., & Pauli, P. (2010). A rift between implicit and explicit conditioned valence in human pain relief learning. *Proc Biol Sci*, *277*(1692), 2411–6. Journal Article. http://doi.org/10.1098/rspb.2010.0103

Andreatta, M., Neueder, D., Genheimer, H., Schiele, M. A., Schartner, C., Deckert, J., … Pauli, P. (2019). Human bdnf rs6265 polymorphism as a mediator for the generalization of contextual anxiety. *J Neurosci Res*, *97*(3), 300–312. Journal Article. http://doi.org/10.1002/jnr.24345

Andreatta, M., Neueder, D., Herzog, K., Genheimer, H., Schiele, M. A., Deckert, J., … Pauli, P. (2020). Generalization of conditioned contextual anxiety and the modulatory effects of anxiety sensitivity. *Neurotherapeutics*.

Antov, M. I., Plog, E., Bierwirth, P., Keil, A., & Stockhorst, U. (2020). Visuocortical tuning to a threat-related feature persists after extinction and consolidation of conditioned fear. *Scientific Reports*, *10*(1), 3926.

Arnaudova, I., Krypotos, A. M., Effting, M., Boddez, Y., Kindt, M., & Beckers, T. (2013). Individual differences in discriminatory fear learning under conditions of ambiguity: A vulnerability factor for anxiety disorders? *Front Psychol*, *4*, 298. Journal Article. `http://doi.org/10.3389/fpsyg.2013.00298`

Askew, C., & Field, A. P. (2007). Vicarious learning and the development of fears in childhood. *Behaviour Research and Therapy*, *45*(11), 2616–2627. `http://doi.org/https://doi.org/10.1016/j.brat.2007.06.008`

Avery, S. N., Clauss, J. A., Winder, D. G., Woodward, N., Heckers, S., & Blackford, J. U. (2014). BNST neurocircuitry in humans. *Neuroimage*, *91*, 311–23. Journal Article. `http://doi.org/10.1016/j.neuroimage.2014.01.017`

Baas, J. M., Ooijen, L. van, Goudriaan, A., & Kenemans, J. L. (2008). Failure to condition to a cue is associated with sustained contextual fear. *Acta Psychol (Amst)*, *127*(3), 581–92. Journal Article. `http://doi.org/10.1016/j.actpsy.2007.09.009`

Bach, D. R., Flandin, G., Friston, K. J., & Dolan, R. J. (2010). Modelling event-related skin conductance responses. *International Journal of Psychophysiology*, *75*(3), 349–356.

Bantin, T., Stevens, S., Gerlach, A. L., & Hermann, C. (2016). What does the facial dot-probe task tell us about attentional processes in social anxiety? A systematic review. *Journal of Behavior Therapy and Experimental Psychiatry*, *50*, 40–51. Journal Article. `http://doi.org/https://doi.org/10.1016/j.jbtep.2015.04.009`

Bar-Haim, Y., Lamy, D., Pergamin, L., Bakermans-Kranenburg, M. J., & van, I. M. H. (2007). Threat-related attentional bias in anxious and nonanxious individuals: A meta-analytic study. *Psychol Bull*, *133*(1), 1–24. Journal Article. `http://doi.org/10.1037/0033-2909.133.1.1`

Barlow, D. H. (2004). *Anxiety and its disorders: The nature and treatment of anxiety and panic*. Guilford press.

Barry-Anwar, R., Hadley, H., Conte, S., Keil, A., & Scott, L. S. (2018). The developmental time course and topographic distribution of individual-level monkey face discrimination in the infant brain. *Neuropsychologia*, *108*, 25–31. Journal Article. `http://doi.org/`

https://doi.org/10.1016/j.neuropsychologia.2017.11.019

Baxter, A. J., Scott, K. M., Vos, T., & Whiteford, H. A. (2013). Global prevalence of anxiety disorders: A systematic review and meta-regression. *Psychological Medicine*, *43*(5), 897–910. http://doi.org/10.1017/S003329171200147X

Beckers, T., Krypotos, A. M., Boddez, Y., Effting, M., & Kindt, M. (2013). What's wrong with fear conditioning? *Biol Psychol*, *92*(1), 90–6. Journal Article. http://doi.org/10.1016/j.biopsycho.2011.12.015

Bekhtereva, V., & Müller, M. M. (2017). Corrigendum to: Affective facilitation of early visual cortex during rapid picture presentation at 6 and 15 hz. *Soc Cogn Affect Neurosci*, *12*(6), 1022–1023.

Bekhtereva, V., Pritschmann, R., Keil, A., & Müller, M. M. (2018). The neural signature of extracting emotional content from rapid visual streams at multiple presentation rates: A cross-laboratory study, *55*(12), e13222. Journal Article. http://doi.org/doi:10.1111/psyp.13222

Blair, H. T., Schafe, G. E., Bauer, E. P., Rodrigues, S. M., & LeDoux, J. E. (n.d.). Synaptic plasticity in the lateral amygdala: A cellular hypothesis of fear conditioning. *Learning & Memory*, *8*(5), 229–242. http://doi.org/10.1101/lm.30901

Blanchard, D. C., Hynd, A. L., Minke, K. A., Minemoto, T., & Blanchard, R. J. (2001). Human defensive behaviors to threat scenarios show parallels to fear- and anxiety-related defense patterns of non-human mammals. *Neurosci Biobehav Rev*, *25*(7-8), 761–70. Journal Article.

Blanchard, R. J., & Blanchard, D. (1989). Attack and defense in rodents as ethoexperimental models for the study of emotion. *Progress in Neuro-Psychopharmacology and Biological Psychiatry*, *13*, S3–S14. http://doi.org/https://doi.org/10.1016/0278-5846(89)90105-X

Bolles, R. C. (1970). Species-specific defense reactions and avoidance learning. *Psychological Review*, *77*(1), 32–48. http://doi.org/10.1037/h0028589

Boucsein, W., Fowles, D. C., Grimnes, S., Ben-Shakhar, G., roth, W. T., Dawson, M. E., … Society for Psychophysiological Research Ad Hoc Committee on Electrodermal, M. (2012). Publication recommendations for electrodermal measurements. *Psychophysiology*, *49*(8), 1017–34. Journal Article. http://doi.org/10.1111/j.1469-8986.2012.01384.x

Bouton, M. E. (2004). Context and behavioral processes in extinction. *Learn Mem*, *11*(5), 485–94. Journal Article. http://doi.org/10.1101/lm.78804

Boylan, M. R., Kelly, M. N., Thigpen, N. N., & Keil, A. (2019). Attention to a threat-related feature does not interfere with concurrent attentive feature selection. *Psychophysiology*, *0*(0), e13332. Journal Article. http://doi.org/10.1111/psyp.13332

Bradley, B. P., Mogg, K., Falla, S. J., & Hamilton, L. R. (1998). Attentional bias for threatening facial expressions in anxiety: Manipulation of stimulus duration. *Cognition and Emotion*, *12*(6), 737–753. http://doi.org/10.1080/026999398379411

Bradley, M. M., Keil, A., & Lang, P. J. (2012). Orienting and emotional perception: Facilitation, attenuation, and interference. *Front Psychol*, *3*, 493. Journal Article. http://doi.org/10.3389/fpsyg.2012.00493

Bradley, M. M., & Lang, P. J. (2000). Measuring emotion: Behavior, feeling, and physiology. In R. D. R. Lane, L. Nadel, G. L. Ahern, J. Allen, & A. W. Kaszniak (Eds.), *Cognitive neuroscience of emotion* (pp. 25–49). Book Section, Oxford University Press.

Bradley, M. M., Moulder, B., & Lang, P. J. (2005). When good things go bad - the reflex physiology of defense. *Psychological Science*, *16*(6), 468–473. Journal Article.

Brosch, T., & Sharma, D. (2005). The role of fear-relevant stimuli in visual search: A comparison of phylogenetic and ontogenetic stimuli. *Emotion*, *5*(3), 360.

Bublatzky, F., Flaisch, T., Stockburger, J., Schmalzle, R., & Schupp, H. T. (2010). The interaction of anticipatory anxiety and emotional picture processing: An event-related brain potential study. *Psychophysiology*, *47*(4), 687–96. Journal Article. http://doi.org/10.1111/j.1469-8986.2010.00966.x

Bublatzky, F., Gerdes, A. B., & Alpers, G. W. (2014). The persistence of socially instructed threat: Two threat-of-shock studies. *Psychophysiology*, *51*(10), 1005–14. Journal Article. http://doi.org/10.1111/psyp.12251

Bublatzky, F., Guerra, P., & Alpers, G. W. (2018). Verbal instructions override the meaning of facial expressions. *Sci Rep*, *8*(1), 14988. Journal Article. http://doi.org/10.1038/s41598-018-33269-2

Bublatzky, F., Guerra, P. M., Pastor, M. C., Schupp, H. T., & Vila, J. (2013). Additive effects of threat-of-shock and picture valence on startle reflex modulation. *PLoS One*, *8*(1), e54003. Journal Article. http://doi.org/10.1371/journal.pone.0054003

Buchel, C., & Dolan, R. J. (2000). Classical fear conditioning in functional neuroimaging. *Curr Opin Neurobiol*, *10*(2), 219–23. Journal Article.

Buhr, K., & Dugas, M. J. (2002). The intolerance of uncertainty scale: Psychometric properties of the english version. *Behav Res Ther*, *40*(8), 931–45. Journal Article.

Butler, A. C., Chapman, J. E., Forman, E. M., & Beck, A. T. (2006). The empirical status of cognitive-behavioral therapy: A review of meta-analyses. *Clinical Psychology Review*, *26*(1), 17–31.

Campagnoli, R. R., Wieser, M. J., Gruss, L. F., Boylan, M. R., McTeague, L. M., & Keil, A. (2019). How the visual brain detects emotional changes in facial expressions: Evidence from driven and intrinsic brain oscillations. *Cortex*, *111*, 35–50.

Carandini, M., Demb, J. B., Mante, V., Tolhurst, D. J., Dan, Y., Olshausen, B. A., … Rust, N. C. (2005). Do we know what the early visual system does? *The Journal of Neuroscience*, *25*(46), 10577–10597. http://doi.org/10.1523/JNEUROSCI.3726-05.2005

Carleton, R. N. (2016). Fear of the unknown: One fear to rule them all? *J Anxiety Disord*, *41*, 5–21. Journal Article. http://doi.org/10.1016/j.janxdis.2016.03.011

Carpenter, J. K., Andrews, L. A., Witcraft, S. M., Powers, M. B., Smits, J. A. J., & Hofmann, S. G. (2018). Cognitive behavioral therapy for anxiety and related disorders: A meta-analysis of randomized placebo-controlled trials. *Depress Anxiety*, *35*(6), 502–514. Journal Article. http://doi.org/10.1002/da.22728

Carrasco, M. (2011). Visual attention: The past 25 years. *Vision Research*, *51*(13), 1484–1525.

Chambers, J. A., Power, K. G., & Durham, R. C. (2004). The relationship between trait vulnerability and anxiety and depressive diagnoses at long-term follow-up of generalized anxiety disorder. *J Anxiety Disord*, *18*(5), 587–607. Journal Article. http://doi.org/10.1016/j.janxdis.2003.09.001

Chen, Y. P., Ehlers, A., Clark, D. M., & Mansell, W. (2002). Patients with generalized social phobia direct their attention away from faces. *Behaviour Research and Therapy*, *40*(6), 677–687.

Cisler, J. M., & Koster, E. H. (2010). Mechanisms of attentional biases towards threat in anxiety disorders: An integrative review. *Clin Psychol Rev*, *30*(2), 203–16. Journal Article. http://doi.org/10.1016/j.cpr.2009.11.003

Croft, R. J., & Barry, R. J. (2000). Removal of ocular artifact from the eeg: A review. *Neurophysiologie Clinique/Clinical Neurophysiology*, *30*(1), 5–19.

Cuthbert, B. N. (2014). The rdoc framework: Facilitating transition from icd/dsm to dimensional approaches that integrate neuroscience and psychopathology. *World Psychiatry*, *13*(1), 28–35. Journal Article. http://doi.org/10.1002/wps.20087

Davis, M. (1992). The role of the amygdala in fear and anxiety. *Annu. Rev. Neurosci.*, *15*(1), 353–375. http://doi.org/10.1146/annurev.ne.15.030192.002033

Davis, M., Walker, D. L., & Lee, Y. (1997). Amygdala and bed nucleus of the stria terminalis: Differential roles in fear and anxiety measured with the acoustic startle reflex. *Philos Trans R Soc Lond B Biol Sci*, *352*(1362), 1675–87. Journal Article. http://doi.org/10.1098/rstb.1997.0149

Davis, M., Walker, D. L., Miles, L., & Grillon, C. (2010). Phasic vs sustained fear in rats and humans: Role of the extended amygdala in fear vs anxiety. *Neuropsychopharmacology*, *35*(1), 105–35. Journal Article. http://doi.org/10.1038/npp.2009.109

Di Russo, F., Pitzalis, S., Aprile, T., Spitoni, G., Patria, F., Stella, A., … Hillyard, S. A. (2007). Spatiotemporal analysis of the cortical sources of the steady-state visual evoked potential. *Hum Brain Mapp*, *28*(4), 323–34. Journal Article. http://doi.org/10.1002/hbm.20276

Dresler, T., Ehlis, A.-C., Hindi Attar, C., Ernst, L. H., Tupak, S. V., Hahn, T., … Fallgatter, A. J. (2012). Reliability of the emotional stroop task: An investigation of patients with panic disorder. *Journal of Psychiatric Research*, *46*(9), 1243–1248.

Duits, P., Cath, D. C., Lissek, S., Hox, J. J., Hamm, A. O., Engelhard, I. M., … Baas, J. M. (2015). Updated meta-analysis of classical fear conditioning in the anxiety disorders. *Depress Anxiety*, *32*(4), 239–53. Journal Article. http://doi.org/10.1002/da.22353

Etkin, A., & Wager, T. D. (2007). Functional neuroimaging of anxiety: A meta-analysis of emotional processing in ptsd, social anxiety disorder, and specific phobia. *Am J Psychiatry*, *164*(10), 1476–88. Journal Article. http://doi.org/10.1176/appi.ajp.2007.07030504

Eysenck, M. W., Derakshan, N., Santos, R., & Calvo, M. G. (2007). Anxiety and cognitive performance: Attentional control theory. *Emotion*, *7*(2), 336.

Fanselow, M. S. (1994). Neural organization of the defensive behavior system responsible for fear. *Psychon Bull Rev*, *1*(4), 429–38. Journal Article. http://doi.org/10.3758/BF03210947

Fanselow, M. S. (2018). The role of learning in threat imminence and defensive behaviors. *Current Opinion in Behavioral Sciences*, *24*, 44–49. Journal Article. http://doi.org/https://doi.org/10.1016/j.cobeha.2018.03.003

Fanselow, M. S., & Lester, L. S. (1988). A functional behavioristic approach to aversively motivated behavior: Predatory imminence as a determinant of the topography of defensive behavior. In *Evolution and learning.* (pp. 185–212). Hillsdale, NJ, US: Lawrence

Erlbaum Associates, Inc.

Fox, A. S., Oler, J. A., Tromp do, P. M., Fudge, J. L., & Kalin, N. H. (2015). Extending the amygdala in theories of threat processing. *Trends Neurosci*, *38*(5), 319–29. Journal Article. http://doi.org/10.1016/j.tins.2015.03.002

Fox, A. S., & Shackman, A. J. (2019). The central extended amygdala in fear and anxiety: Closing the gap between mechanistic and neuroimaging research. *Neurosci Lett*, *693*, 58–67. Journal Article. http://doi.org/10.1016/j.neulet.2017.11.056

Fox, E., Russo, R., Bowles, R., & Dutton, K. (2001). Do threatening stimuli draw or hold visual attention in subclinical anxiety? *Journal of Experimental Psychology: General*, *130*(4), 681.

Fullana, M. A., Albajes-Eizagirre, A., Soriano-Mas, C., Vervliet, B., Cardoner, N., Benet, O., … Harrison, B. J. (2018). Fear extinction in the human brain: A meta-analysis of fMRI studies in healthy participants. *Neurosci Biobehav Rev*, *88*, 16–25. Journal Article. http://doi.org/10.1016/j.neubiorev.2018.03.002

Fullana, M. A., Harrison, B. J., Soriano-Mas, C., Vervliet, B., Cardoner, N., Avila-Parcet, A., & Radua, J. (2016). Neural signatures of human fear conditioning: An updated and extended meta-analysis of fMRI studies. *Mol Psychiatry*, *21*(4), 500–8. Journal Article. http://doi.org/10.1038/mp.2015.88

Genheimer, H., Andreatta, M., & Pauli, P. (2020). Conjunctive and elemental representations of a context in humans. *Journal of Cognitive Neuroscience*, 1–13. http://doi.org/10.1162/jocn_a_01559

Gilbert, C. D., & Sigman, M. (2007). Brain states: Top-down influences in sensory processing. *Neuron*, *54*(5), 677–96. Journal Article. http://doi.org/10.1016/j.neuron.2007.05.019

Gilbert, C. D., Sigman, M., & Crist, R. E. (2001). The neural basis of perceptual learning. *Neuron*, *31*(5), 681–697. http://doi.org/10.1016/s0896-6273(01)00424-x

Glotzbach, E., Ewald, H., Andreatta, M., Pauli, P., & Muhlberger, A. (2012). Contextual fear conditioning predicts subsequent avoidance behaviour in a virtual reality environment. *Cogn Emot*, *26*(7), 1256–72. Journal Article. http://doi.org/10.1080/02699931.2012.656581

Glotzbach, E., Ewald, H., Andreatta, M., Pauli, P., & Mühlberger, A. (2012). Contextual fear conditioning predicts subsequent avoidance behaviour in a virtual reality environment. *Cognition and Emotion*, *26*(7), 1256–1272. http://doi.org/10.1080/02699931.2012.656581

Glotzbach-Schoon, E., Andreatta, M., Muhlberger, A., & Pauli, P. (2015). Reinstatement of contextual anxiety in humans: Effects of state anxiety. *Int J Psychophysiol*, *98*(3 Pt 2), 557–66. Journal Article. http://doi.org/10.1016/j.ijpsycho.2015.07.013

Glotzbach-Schoon, E., Andreatta, M., Reif, A., Ewald, H., Troger, C., Baumann, C., … Pauli, P. (2013). Contextual fear conditioning in virtual reality is affected by 5HTTLPR and npsr1 polymorphisms: Effects on fear-potentiated startle. *Front Behav Neurosci*, *7*, 31. Journal Article. http://doi.org/10.3389/fnbeh.2013.00031

Glotzbach-Schoon, E., Tadda, R., Andreatta, M., Troger, C., Ewald, H., Grillon, C., … Muhlberger, A. (2013). Enhanced discrimination between threatening and safe contexts in high-anxious individuals. *Biol Psychol*, *93*(1), 159–66. Journal Article. http://doi.org/10.1016/j.biopsycho.2013.01.011

Goodale, M. A., & Milner, A. D. (1992). Separate visual pathways for perception and action. *Trends in Neurosciences*, *15*(1), 20–25.

Gorka, S. M., Lieberman, L., Shankman, S. A., & Phan, K. L. (2017a). Association between neural reactivity and startle reactivity to uncertain threat in two independent samples. *Psychophysiology*, *54*(5), 652–662. Journal Article. http://doi.org/10.1111/psyp.12829

Gorka, S. M., Lieberman, L., Shankman, S. A., & Phan, K. L. (2017b). Startle potentiation to uncertain threat as a psychophysiological indicator of fear-based psychopathology: An examination across multiple internalizing disorders. *J Abnorm Psychol*, *126*(1), 8–18. Journal Article. http://doi.org/10.1037/abn0000233

Gorka, S. M., Nelson, B. D., & Shankman, S. A. (2013). Startle response to unpredictable threat in comorbid panic disorder and alcohol dependence. *Drug Alcohol Depend*, *132*(1-2), 216–22. Journal Article. http://doi.org/10.1016/j.drugalcdep.2013.02.003

Graham, B. M., & Milad, M. R. (2011). The study of fear extinction: Implications for anxiety disorders. *The American Journal of Psychiatry*, *168*(21865528), 1255–1265.

Greenhouse, S., & Geisser, S. (1959). On methods in the analysis of profile data. *Psychometrika*, *24*(2), 95–112. Journal Article.

Grillon, C. (2002). Startle reactivity and anxiety disorders: Aversive conditioning, context, and neurobiology. *Biol Psychiatry*, *52*(10), 958–75. Journal Article.

Grillon, C., Ameli, R., Woods, S. W., Merikangas, K., & Davis, M. (1991). Fear-potentiated startle in humans: Effects of anticipatory anxiety on the acoustic blink reflex. *Psychophysiology*, *28*(5), 588–595. http://doi.org/10.1111/j.1469-8986.1991.tb01999.x

Grillon, C., Baas, J. M., Cornwell, B., & Johnson, L. (2006). Context conditioning and behavioral avoidance in a virtual reality environment: Effect of predictability. *Biol Psychiatry*, *60*(7), 752–9. Journal Article. http://doi.org/10.1016/j.biopsych.2006.03.072

Grillon, C., Baas, J. P., Lissek, S., Smith, K., & Milstein, J. (2004). Anxious responses to predictable and unpredictable aversive events. *Behav Neurosci*, *118*(5), 916–24. Journal Article. http://doi.org/10.1037/0735-7044.118.5.916

Grillon, C., Franco-Chaves, J. A., Mateus, C. F., Ionescu, D. F., & Zarate, C. A. (2013). Major depression is not associated with blunting of aversive responses; evidence for enhanced anxious anticipation. *PLoS One*, *8*(8), e70969. Journal Article. http://doi.org/10.1371/journal.pone.0070969

Grillon, C., Lissek, S., Rabin, S., McDowell, D., Dvir, S., & Pine, D. S. (2008). Increased anxiety during anticipation of unpredictable but not predictable aversive stimuli as a psychophysiologic marker of panic disorder. *Am J Psychiatry*, *165*(7), 898–904. Journal Article. http://doi.org/10.1176/appi.ajp.2007.07101581

Grillon, C., Pine, D. S., Lissek, S., Rabin, S., Bonne, O., & Vythilingam, M. (2009). Increased anxiety during anticipation of unpredictable aversive stimuli in posttraumatic stress disorder but not in generalized anxiety disorder. *Biol Psychiatry*, *66*(1), 47–53. Journal Article. http://doi.org/10.1016/j.biopsych.2008.12.028

Grillon, C., Robinson, O. J., Cornwell, B., & Ernst, M. (2019). Modeling anxiety in healthy humans: A key intermediate bridge between basic and clinical sciences. *Neuropsychopharmacology*. Journal Article. http://doi.org/10.1038/s41386-019-0445-1

Grupe, D. W., & Nitschke, J. B. (2013). Uncertainty and anticipation in anxiety: An integrated neurobiological and psychological perspective. *Nat Rev Neurosci*, *14*(7), 488–501. Journal Article. http://doi.org/10.1038/nrn3524

Gruss, L. F., Wieser, M. J., Schweinberger, S., & Keil, A. (n.d.). Face-evoked steady-state visual potentials: Effects of presentation rate and face inversion. *Frontiers in Human Neuroscience*. http://doi.org/10.3389/fnhum.2012.00316

Haaker, J., Golkar, A., Selbing, I., & Olsson, A. (2017). Assessment of social transmission of threats in humans using observational fear conditioning. *Nature Protocols*, *12*(7), 1378–1386.

Haaker, J., Lonsdorf, T. B., Thanellou, A., & Kalisch, R. (2013). Multimodal assessment of long-term memory recall and reinstatement in a combined cue and context fear conditioning and extinction paradigm in humans. *PLoS One*, *8*(10), e76179. Journal Article.

http://doi.org/10.1371/journal.pone.0076179

Haaker, J., Maren, S., Andreatta, M., Merz, C. J., Richter, J., Richter, S. H., … Lonsdorf, T. B. (2019). Making translation work: Harmonizing cross-species methodology in the behavioural neuroscience of pavlovian fear conditioning. *Neuroscience & Biobehavioral Reviews*, *107*, 329–345.

Haddad, A. D. M., Pritchett, D., Lissek, S., & Lau, J. Y. F. (2012). Trait anxiety and fear responses to safety cues: Stimulus generalization or sensitization? *Journal of Psychopathology and Behavioral Assessment*, *34*(3), 323–331. Journal Article. http://doi.org/10.1007/s10862-012-9284-7

Hamm, A. O. (2020). Fear, anxiety, and their disorders from the perspective of psychophysiology. *Psychophysiology*, *57*(2), e13474. http://doi.org/10.1111/psyp.13474

Hebb, D. O. (1949). *The organization of behavior: A neuropsychological theory*. J. Wiley; Chapman & Hall.

Herrmann, M. J., Boehme, S., Becker, M. P., Tupak, S. V., Guhn, A., Schmidt, B., … Straube, T. (2016). Phasic and sustained brain responses in the amygdala and the bed nucleus of the stria terminalis during threat anticipation. *Hum Brain Mapp*, *37*(3), 1091–102. Journal Article. http://doi.org/10.1002/hbm.23088

Hitchcock, J. M., & Davis, M. (1991). Efferent pathway of the amygdala involved in conditioned fear as measured with the fear-potentiated startle paradigm. *Behavioral Neuroscience*, *105*(6), 826.

Hofmann, S. G. (2008). Cognitive processes during fear acquisition and extinction in animals and humans: Implications for exposure therapy of anxiety disorders. *Clinical Psychology Review*, *28*(2), 199–210. http://doi.org/https://doi.org/10.1016/j.cpr.2007.04.009

Hofmann, S. G., & Smits, J. A. J. (2008). Cognitive-behavioral therapy for adult anxiety disorders: A meta-analysis of randomized placebo-controlled trials. *The Journal of Clinical Psychiatry*, *69*(18363421), 621–632.

Horton, J. C., & Hoyt, W. F. (1991). The representation of the visual field in human striate cortex: A revision of the classic holmes map. *Archives of Ophthalmology*, *109*(6), 816–824. http://doi.org/10.1001/archopht.1991.01080060080030

Huffmeijer, R., Bakermans-Kranenburg, M. J., Alink, L. R. A., & Ijzendoorn, M. H. van. (2014). Reliability of event-related potentials: The influence of number of trials and electrodes. *Physiology & Behavior*, *130*(24642000), 13–22.

Hur, J., Stockbridge, M. D., Fox, A. S., & Shackman, A. J. (2019). Dispositional negativity, cognition, and anxiety disorders: An integrative translational neuroscience framework. *Progress in Brain Research*, *247*, 375–436. Journal Article. http://doi.org/10.1016/bs.pbr.2019.03.012

Insel, T., Cuthbert, B., Garvey, M., Heinssen, R., Pine, D. S., Quinn, K., … Wang, P. (2010). Research domain criteria (rdoc): Toward a new classification framework for research on mental disorders. *Am J Psychiatry*, *167*(7), 748–51. Journal Article. http://doi.org/10.1176/appi.ajp.2010.09091379

Insel, T. R. (2014). The nimh research domain criteria (rdoc) project: Precision medicine for psychiatry. *Am J Psychiatry*, *171*(4), 395–7. Journal Article. http://doi.org/10.1176/appi.ajp.2014.14020138

Junghofer, M., Elbert, T., Tucker, D. M., & Rockstroh, B. (2000). Statistical control of artifacts in dense array eeg/meg studies. *Psychophysiology*, *37*(4), 523–32. Journal Article.

Junghöfer, M., Elbert, T., Leiderer, P., Berg, P., & Rockstroh, B. (1997). Mapping eeg-potentials on the surface of the brain: A strategy for uncovering cortical sources. *Brain Topography*, *9*(3), 203–217. Journal Article. http://doi.org/10.1007/BF01190389

Kastner, A. K., Pauli, P., & Wieser, M. J. (2015). Sustained attention in context conditioning: Evidence from steady-state veps. *Int J Psychophysiol*, *98*(3 Pt 2), 546–56. Journal Article. http://doi.org/10.1016/j.ijpsycho.2015.03.005

Kastner-Dorn, A. K., Andreatta, M., Pauli, P., & Wieser, M. J. (2018). Hypervigilance during anxiety and selective attention during fear: Using steady-state visual evoked potentials (ssVEPs) to disentangle attention mechanisms during predictable and unpredictable threat. *Cortex*, *106*, 120–131. Journal Article. http://doi.org/10.1016/j.cortex.2018.05.008

Keil, A., Gruber, T., Muller, M. M., Moratti, S., Stolarova, M., Bradley, M. M., & Lang, P. J. (2003). Early modulation of visual perception by emotional arousal: Evidence from steady-state visual evoked brain potentials. *Cogn Affect Behav Neurosci*, *3*(3), 195–206. Journal Article.

Keil, A., Miskovic, V., Gray, M. J., & Martinovic, J. (2013). Luminance, but not chromatic visual pathways mediate amplification of conditioned danger signals in human visual cortex. *The European Journal of Neuroscience*, *38*(9), 10.1111/ejn.12316. Journal Article.

Keil, A., Moratti, S., Sabatinelli, D., Bradley, M. M., & Lang, P. J. (2005). Additive effects of emotional content and spatial selective attention on electrocortical facilitation. *Cereb*

Cortex, *15*(8), 1187–97. Journal Article. http://doi.org/10.1093/cercor/bhi001

Keil, A., Smith, J. C., Wangelin, B. C., Sabatinelli, D., Bradley, M. M., & Lang, P. J. (2008). Electrocortical and electrodermal responses covary as a function of emotional arousal: A single-trial analysis. *Psychophysiology*, *45*(4), 516–23. Journal Article. http://doi.org/10.1111/j.1469-8986.2008.00667.x

Keil, A., Stolarova, M., Moratti, S., & Ray, W. J. (2007). Adaptation in human visual cortex as a mechanism for rapid discrimination of aversive stimuli. *Neuroimage*, *36*(2), 472–9. Journal Article. http://doi.org/10.1016/j.neuroimage.2007.02.048

Klinke, C. M., Fiedler, D., Lange, M. D., & Andreatta, M. (2020). Evidence for impaired extinction learning in humans after distal stress exposure. *Neurobiology of Learning and Memory*, *167*, 107127.

Kruijt, A.-W., Parsons, S., & Fox, E. (2019). A meta-analysis of bias at baseline in rcts of attention bias modification: No evidence for dot-probe bias towards threat in clinical anxiety and ptsd. *Journal of Abnormal Psychology*, *128*(6), 563.

Kuhn, M., Haaker, J., Glotzbach-Schoon, E., Schumann, D., Andreatta, M., Mechias, M. L., … Lonsdorf, T. B. (2016). Converging evidence for an impact of a functional nos gene variation on anxiety-related processes. *Soc Cogn Affect Neurosci*, *11*(5), 803–12. Journal Article. http://doi.org/10.1093/scan/nsv151

Lamme, V. A. F., Supèr, H., & Spekreijse, H. (1998). Feedforward, horizontal, and feedback processing in the visual cortex. *Current Opinion in Neurobiology*, *8*(4), 529–535.

Lang, P. J., & Bradley, M. M. (2010). Emotion and the motivational brain. *Biological Psychology*, *84*(3), 437–450. Journal Article. http://doi.org/https://doi.org/10.1016/j.biopsycho.2009.10.007

Lang, P. J., Bradley, M. M., & Cuthbert, B. N. (1997). Motivated attention: Affect, activation, and action. *Attention and Orienting: Sensory and Motivational Processes*, *97*, 135.

Lang, P. J., Davis, M., & Ohman, A. (2000). Fear and anxiety: Animal models and human cognitive psychophysiology. *J Affect Disord*, *61*(3), 137–59. Journal Article.

Lang, S., Kroll, A., Lipinski, S. J., Wessa, M., Ridder, S., Christmann, C., … Flor, H. (2009). Context conditioning and extinction in humans: Differential contribution of the hippocampus, amygdala and prefrontal cortex. *Eur J Neurosci*, *29*(4), 823–32. Journal Article. http://doi.org/10.1111/j.1460-9568.2009.06624.x

Laux, L., & Spielberger, C. D. (1981). *Das state-trait-angstinventar: STAI*. Book, Beltz

Weinheim.

LeDoux, J. (2012). Rethinking the emotional brain. *Neuron*, *73*(22365542), 653–676.

LeDoux, J. E. (1996). *The emotional brain: The mysterious underpinnings of emotional life*. Book, New York, NY US: Simon & Schuster.

LeDoux, J. E. (2000). Emotion circuits in the brain. *Annual Review of Neuroscience*, *23*(1), 155–184. http://doi.org/10.1146/annurev.neuro.23.1.155

LeDoux, J. E. (2014). Coming to terms with fear. *Proceedings of the National Academy of Sciences*, *111*(8), 2871–2878. http://doi.org/10.1073/pnas.1400335111

LeDoux, J. E., & Pine, D. S. (2016). Using neuroscience to help understand fear and anxiety: A two-system framework. *Am J Psychiatry*, *173*(11), 1083–1093. Journal Article. http://doi.org/10.1176/appi.ajp.2016.16030353

Le Pelley, M. E. (2004). The role of associative history in models of associative learning: A selective review and a hybrid model. *The Quarterly Journal of Experimental Psychology Section B*, *57*(3b), 193–243. http://doi.org/10.1080/02724990344000141

Lesting, J., Narayanan, R. T., Kluge, C., Sangha, S., Seidenbecher, T., & Pape, H.-C. (2011). Patterns of coupled theta activity in amygdala-hippocampal-prefrontal cortical circuits during fear extinction. *PLOS ONE*, *6*(6), e21714. http://doi.org/10.1371/journal.pone.0021714

Levy, I., & Schiller, D. (2021). Neural computations of threat. *Trends in Cognitive Sciences*, *25*(2), 151–171.

Li, W. (2014). Learning to smell danger: Acquired associative representation of threat in the olfactory cortex. *Frontiers in Behavioral Neuroscience*, *8*, 98.

Lissek, S., Powers, A. S., McClure, E. B., Phelps, E. A., Woldehawariat, G., Grillon, C., & Pine, D. S. (2005). Classical fear conditioning in the anxiety disorders: A meta-analysis. *Behav Res Ther*, *43*(11), 1391–424. Journal Article. http://doi.org/10.1016/j.brat.2004.10.007

Loerinc, A. G., Meuret, A. E., Twohig, M. P., Rosenfield, D., Bluett, E. J., & Craske, M. G. (2015). Response rates for cbt for anxiety disorders: Need for standardized criteria. *Clinical Psychology Review*, *42*, 72–82.

Lommen, M. J. J., Engelhard, I. M., Sijbrandij, M., Hout, M. A. van den, & Hermans, D. (2013). Pre-trauma individual differences in extinction learning predict posttraumatic stress. *Behaviour Research and Therapy*, *51*(2), 63–67.

Lonsdorf, T. B., Haaker, J., & Kalisch, R. (2014). Long-term expression of human contextual fear and extinction memories involves amygdala, hippocampus and ventromedial prefrontal cortex: A reinstatement study in two independent samples. *Soc Cogn Affect Neurosci*, *9*(12), 1973–83. Journal Article. http://doi.org/10.1093/scan/nsu018

Lonsdorf, T. B., Klingelhöfer-Jens, M., Andreatta, M., Beckers, T., Chalkia, A., Gerlicher, A., … Merz, C. J. (2019). Navigating the garden of forking paths for data exclusions in fear conditioning research. *eLife*, *8*, e52465. http://doi.org/10.7554/eLife.52465

Lonsdorf, T. B., Menz, M. M., Andreatta, M., Fullana, M. A., Golkar, A., Haaker, J., … Merz, C. J. (2017). Don't fear 'fear conditioning': Methodological considerations for the design and analysis of studies on human fear acquisition, extinction, and return of fear. *Neurosci Biobehav Rev*, *77*, 247–285. Journal Article. http://doi.org/10.1016/j.neubiorev.2017.02.026

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

Lovibond, P. F., & Lovibond, S. H. (1995). The structure of negative emotional states: Comparison of the depression anxiety stress scales (dass) with the beck depression and anxiety inventories. *Behav Res Ther*, *33*(3), 335–43. Journal Article. http://doi.org/http://dx.doi.org/10.1016/0005-7967(94)00075-U

Lovibond, P. F., & Shanks, D. R. (2002). The role of awareness in pavlovian conditioning: Empirical evidence and theoretical implications. *J Exp Psychol Anim Behav Process*, *28*(1), 3–26. Journal Article.

MacLeod, C., Mathews, A., & Tata, P. (1986). Attentional bias in emotional disorders. *Journal of Abnormal Psychology*, *95*(1), 15.

MacLeod, C., & Rutherford, E. M. (1992). Anxiety and the selective processing of emotional information: Mediating roles of awareness, trait and state variables, and personal relevance of stimulus materials. *Behav Res Ther*, *30*(5), 479–91. Journal Article.

Maren, S., Phan, K. L., & Liberzon, I. (2013). The contextual brain: Implications for fear conditioning, extinction and psychopathology. *Nat Rev Neurosci*, *14*(6), 417–28. Journal Article. http://doi.org/10.1038/nrn3492

Mathews, A., & Mackintosh, B. (1998). A cognitive model of selective processing in anxiety. *Cognitive Therapy and Research*, *22*(6), 539–560.

Mathews, A., May, J., Mogg, K., & Eysenck, M. (1990). Attentional bias in anxiety: Selective search or defective filtering? *J Abnorm Psychol*, *99*(2), 166–73. Journal Article.

McTeague, L. M., Gruss, L. F., & Keil, A. (2015). Aversive learning shapes neuronal orientation tuning in human visual cortex. *Nat Commun*, *6*, 7823. Journal Article. http://doi.org/10.1038/ncomms8823

McTeague, L. M., Laplante, M. C., Bulls, H. W., Shumen, J. R., Lang, P. J., & Keil, A. (2018). Face perception in social anxiety: Visuocortical dynamics reveal propensities for hypervigilance or avoidance. *Biol Psychiatry*, *83*(7), 618–628. Journal Article. http://doi.org/10.1016/j.biopsych.2017.10.004

McTeague, L. M., Shumen, J. R., Wieser, M. J., Lang, P. J., & Keil, A. (2011). Social vision: Sustained perceptual enhancement of affective facial cues in social anxiety. *Neuroimage*, *54*(2), 1615–24. Journal Article. http://doi.org/10.1016/j.neuroimage.2010.08.080

Mechias, M. L., Etkin, A., & Kalisch, R. (2010). A meta-analysis of instructed fear studies: Implications for conscious appraisal of threat. *Neuroimage*, *49*(2), 1760–8. Journal Article. http://doi.org/10.1016/j.neuroimage.2009.09.040

Merscher, A.-S., & Gamer, M. (2020). Freezing of gaze in anticipation of avoidable threat: A threat-specific proxy for freezing-like behavior in humans. In *ACM symposium on eye tracking research and applications*. New York, NY, USA: Association for Computing Machinery. http://doi.org/10.1145/3379156.3391345

Mertens, G., Boddez, Y., Sevenster, D., Engelhard, I. M., & De Houwer, J. (2018). A review on the effects of verbal instructions in human fear conditioning: Empirical findings, theoretical considerations, and future directions. *Biol Psychol*, *137*, 49–64. Journal Article. http://doi.org/10.1016/j.biopsycho.2018.07.002

Milad, M. R., Orr, S. P., Pitman, R. K., & Rauch, S. L. (2005). Context modulation of memory for fear extinction in humans. *Psychophysiology*, *42*(4), 456–64. Journal Article. http://doi.org/10.1111/j.1469-8986.2005.00302.x

Milad, M. R., & Quirk, G. J. (2012). Fear extinction as a model for translational neuroscience: Ten years of progress. *Annu Rev Psychol*, *63*, 129–51. Journal Article. http://doi.org/10.1146/annurev.psych.121208.131631

Milad, M. R., Wright, C. I., Orr, S. P., Pitman, R. K., Quirk, G. J., & Rauch, S. L. (2007). Recall of fear extinction in humans activates the ventromedial prefrontal cortex and hippocampus in concert. *Biological Psychiatry*, *62*(5), 446–454. http://doi.org/10.1016/j.biopsych.2006.10.011

Mineka, S., & Oehlberg, K. (2008). The relevance of recent developments in classical conditioning to understanding the etiology and maintenance of anxiety disorders. *Acta Psychol (Amst)*, *127*(3), 567–80. Journal Article. http://doi.org/10.1016/j.actpsy.2007.11.007

Mineka, S., & Zinbarg, R. (2006). A contemporary learning theory perspective on the etiology of anxiety disorders: It's not what you thought it was. *Am Psychol*, *61*(1), 10–26. Journal Article. http://doi.org/10.1037/0003-066X.61.1.10

Mishkin, M., Ungerleider, L. G., & Macko, K. A. (1983). Object vision and spatial vision: Two cortical pathways. *Trends in Neurosciences*, *6*, 414–417.

Miskovic, V., & Keil, A. (2012). Acquired fears reflected in cortical sensory processing: A review of electrophysiological studies of human classical conditioning. *Psychophysiology*, *49*(9), 1230–41. Journal Article. http://doi.org/10.1111/j.1469-8986.2012.01398.x

Miskovic, V., & Keil, A. (2013a). Perceiving threat in the face of safety: Excitation and inhibition of conditioned fear in human visual cortex. *J Neurosci*, *33*(1), 72–8. Journal Article. http://doi.org/10.1523/JNEUROSCI.3692-12.2013

Miskovic, V., & Keil, A. (2013b). Visuocortical changes during delay and trace aversive conditioning: Evidence from steady-state visual evoked potentials. *Emotion*, *13*(3), 554–61. Journal Article. http://doi.org/10.1037/a0031323

Miskovic, V., & Keil, A. (2014). Escape from harm: Linking affective vision and motor responses during active avoidance. *Soc Cogn Affect Neurosci*, *9*(12), 1993–2000. Journal Article. http://doi.org/10.1093/scan/nsu013

Mitchell, C. J., De Houwer, J., & Lovibond, P. F. (2009). The propositional nature of human associative learning. *Behav Brain Sci*, *32*(2), 183–98; discussion 198–246. Journal Article. http://doi.org/10.1017/S0140525X09000855

Mobbs, D. (2018). The ethological deconstruction of fear(s). *Current Opinion in Behavioral Sciences*, *24*, 32–37.

Mobbs, D., Adolphs, R., Fanselow, M. S., Barrett, L. F., LeDoux, J. E., Ressler, K., & Tye, K. M. (2019). Viewpoints: Approaches to defining and investigating fear. *Nature Neuroscience*, *22*(8), 1205–1216.

Mobbs, D., Hagan, C. C., Dalgleish, T., Silston, B., & Prévost, C. (2015). The ecology of human fear: Survival optimization and the nervous system. *Frontiers in Neuroscience*, *9*, 55. http://doi.org/10.3389/fnins.2015.00055

Mobbs, D., Headley, D. B., Ding, W., & Dayan, P. (2020). Space, time, and fear: Survival computations along defensive circuits. *Trends in Cognitive Sciences*, *24*(3), 228–241.

Mogg, K., & Bradley, B. P. (1998). A cognitive-motivational analysis of anxiety. *Behav Res Ther*, *36*(9), 809–48. Journal Article.

Moratti, S., Clementz, B. A., Gao, Y., Ortiz, T., & Keil, A. (2007). Neural mechanisms of evoked oscillations: Stability and interaction with transient events. *Hum. Brain Mapp.*, *28*(12), 1318–1333. http://doi.org/10.1002/hbm.20342

Moratti, S., & Keil, A. (2009). Not what you expect: Experience but not expectancy predicts conditioned responses in human visual and supplementary cortex. *Cerebral Cortex*, *19*(12), 2803–2809. Journal Article.

Moratti, S., Keil, A., & Miller, G. A. (2006). Fear but not awareness predicts enhanced sensory processing in fear conditioning. *Psychophysiology*, *43*(2), 216–226. Journal Article.

Morriss, J., Hoare, S., & Reekum, C. M. van. (2018). It's time: A commentary on fear extinction in the human brain using fMRI. *Neurosci Biobehav Rev*, *94*, 321–322. Journal Article. http://doi.org/10.1016/j.neubiorev.2018.06.025

Muller, M. M., Andersen, S. K., & Keil, A. (2008). Time course of competition for visual processing resources between emotional pictures and foreground task. *Cereb Cortex*, *18*(8), 1892–9. Journal Article. http://doi.org/10.1093/cercor/bhm215

Muller, M. M., Andersen, S., Trujillo, N. J., Valdes-Sosa, P., Malinowski, P., & Hillyard, S. A. (2006). Feature-selective attention enhances color signals in early visual areas of the human brain. *Proc Natl Acad Sci U S A*, *103*(38), 14250–4. Journal Article. http://doi.org/10.1073/pnas.0606668103

Muller, M. M., Teder-Salejarvi, W., & Hillyard, S. A. (1998). The time course of cortical facilitation during cued shifts of spatial attention. *Nat Neurosci*, *1*(7), 631–4. Journal Article. http://doi.org/10.1038/2865

Nelson, B. D., Hajcak, G., & Shankman, S. A. (2015a). Event-related potentials to acoustic startle probes during the anticipation of predictable and unpredictable threat. *Psychophysiology*, *52*(7), 887–94. Journal Article. http://doi.org/10.1111/psyp.12418

Nelson, B. D., Hodges, A., Hajcak, G., & Shankman, S. A. (2015b). Anxiety sensitivity and the anticipation of predictable and unpredictable threat: Evidence from the startle response and event-related potentials. *J Anxiety Disord*, *33*, 62–71. Journal Article. http://doi.org/10.1016/j.janxdis.2015.05.003

Norcia, A. M., Appelbaum, L. G., Ales, J. M., Cottereau, B. R., & Rossion, B. (2015). The steady-state visual evoked potential in vision research: A review. *J Vis*, *15*(6), 4. Journal Article. http://doi.org/10.1167/15.6.4

Ojala, K. E., & Bach, D. R. (2020). Measuring learning in human classical threat conditioning: Translational, cognitive and methodological considerations. *Neuroscience & Biobehavioral Reviews*.

Onnis, R., Dadds, M. R., & Bryant, R. A. (2011). Is there a mutual relationship between opposite attentional biases underlying anxiety? *Emotion*, *11*(3), 582–94. Journal Article. http://doi.org/10.1037/a0022019

Öhman, A. (2005). The role of the amygdala in human fear: Automatic detection of threat. *Psychoneuroendocrinology*, *30*(10), 953–958. Journal Article.

Öhman, A., Flykt, A., & Esteves, F. (2001). Emotion drives attention: Detecting the snake in the grass. *Journal of Experimental Psychology: General*, *130*(3), 466.

Öhman, A., & Soares, J. J. (1993). On the automatic nature of phobic fear: Conditioned electrodermal responses to masked fear-relevant stimuli. *Journal of Abnormal Psychology*, *102*(1), 121–132. Journal Article.

Panitz, C., Keil, A., & Mueller, E. M. (2019). Extinction-resistant attention to long-term conditioned threat is indexed by selective visuocortical alpha suppression in humans. *Scientific Reports*, *9*(1), 15809.

Pavlov, I. P. (1927). *Conditioned reflexes: An investigation of the physiological activity of the cerebral cortex. Conditioned reflexes: an investigation of the physiological activity of the cerebral cortex.* (pp. xv, 430–xv, 430). Oxford, England: Oxford Univ. Press.

Perruchet, P. (1985). A pitfall for the expectancy theory of human eyelid conditioning. *Pavlov J Biol Sci*, *20*(4), 163–70. Journal Article.

Perruchet, P., & Pacton, S. (2006). Implicit learning and statistical learning: One phenomenon, two approaches. *Trends Cogn Sci*, *10*(5), 233–8. Journal Article. http://doi.org/10.1016/j.tics.2006.03.006

Perusini, J. N., & Fanselow, M. S. (2015). Neurobehavioral perspectives on the distinction between fear and anxiety. *Learning & Memory*, *22*(9), 417–425. Journal Article. http://doi.org/10.1101/lm.039180.115

Pessoa, L., & Adolphs, R. (2010). Emotion processing and the amygdala: From a 'low road' to 'many roads' of evaluating biological significance. *Nature Reviews. Neuroscience*, *11*(20959860), 773–783.

Peyk, P., De Cesarei, A., & Junghofer, M. (2011). ElectroMagnetoEncephalography software: Overview and integration with other eeg/meg toolboxes. *Comput Intell Neurosci*, *2011*, 861705. Journal Article. http://doi.org/10.1155/2011/861705

Picton, T. W., Bentin, S., Berg, P., Donchin, E., Hillyard, S. A., Johnson, J., R., … Taylor, M. J. (2000). Guidelines for using human event-related potentials to study cognition: Recording standards and publication criteria. *Psychophysiology*, *37*(2), 127–52. Journal Article.

Pittig, A., Berg, L. van den, & Vervliet, B. (2016). The key role of extinction learning in anxiety disorders: Behavioral strategies to enhance exposure-based treatments. *Curr Opin Psychiatry*, *29*(1), 39–47. Journal Article. http://doi.org/10.1097/YCO.0000000000000220

Pittig, A., Treanor, M., LeBeau, R. T., & Craske, M. G. (2018). The role of associative fear and avoidance learning in anxiety disorders: Gaps and directions for future research. *Neuroscience & Biobehavioral Reviews*, *88*, 117–140.

Pizzagalli, D. A., Greischar, L. L., & Davidson, R. J. (2003). Spatio-temporal dynamics of brain mechanisms in aversive classical conditioning: High-density event-related potential and brain electrical tomography analyses. *Neuropsychologia*, *41*(2), 184–194.

Ponder, C. A., Kliethermes, C. L., Drew, M. R., Muller, J., Das, K., Risbrough, V. B., … Palmer, A. A. (2007). Selection for contextual fear conditioning affects anxiety-like behaviors and gene expression. *Genes, Brain and Behavior*, *6*(8), 736–749. http://doi.org/10.1111/j.1601-183x.2007.00306.x

Posner, M. I. (1980). Orienting of attention. *Quarterly Journal of Experimental Psychology*, *32*(1), 3–25.

Regan, D. (1989). *Human brain electrophysiology: Evoked potentials and evoked magnetic fields in science and medicine*. Book, Amsterdam, the Netherlands: Elsevier.

Reiss, S., Peterson, R. A., Gursky, D. M., & McNally, R. J. (1986). Anxiety sensitivity, anxiety frequency and the predictions of fearfulness. *Behaviour Research and Therapy*, *24*(1), 1–8. Journal Article.

Rescorla, R. A., & Wagner, A. R. (1972). A theory of pavlovian conditioning: Variations in the effectiveness of reinforcement and nonreinforcement. Journal Article.

Richards, H. J., Benson, V., Donnelly, N., & Hadwin, J. A. (2014). Exploring the function of selective attention and hypervigilance for threat in anxiety. *Clin Psychol Rev*, *34*(1), 1–13. Journal Article. http://doi.org/10.1016/j.cpr.2013.10.006

Richards, H. J., Hadwin, J. A., Benson, V., Wenger, M. J., & Donnelly, N. (2011). The influence of anxiety on processing capacity for threat detection. *Psychonomic Bulletin & Review*, *18*(5), 883.

Richter, J., Pittig, A., Hollandt, M., & Lueken, U. (2017). Bridging the gaps between basic science and cognitive-behavioral treatments for anxiety disorders in routine care. *Zeitschrift Fur Psychologie-Journal of Psychology*, *225*(3), 252–267. Journal Article. http://doi.org/10.1027/2151-2604/a000309

Riels, K. M., Rocha, H. A., & Keil, A. (2020). No intermodal interference effects of threatening information during concurrent audiovisual stimulation. *Neuropsychologia*, *136*, 107283.

Roelofs, K., Hagenaars, M. A., & Stins, J. (2010). Facing freeze: Social threat induces bodily freeze in humans. *Psychol Sci*, *21*(11), 1575–1581. http://doi.org/10.1177/0956797610384746

Rösler, L., & Gamer, M. (2019). Freezing of gaze during action preparation under threat imminence. *Scientific Reports*, *9*(1), 17215.

Ruiter, C. de, & Brosschot, J. F. (1994). The emotional stroop interference effect in anxiety: Attentional bias or cognitive avoidance? *Behaviour Research and Therapy*, *32*(3), 315–319.

Sabatinelli, D., Lang, P. J., Bradley, M. M., Costa, V. D., & Keil, A. (2009). The timing of emotional discrimination in human amygdala and ventral visual cortex. *The Journal of Neuroscience*, *29*(47), 14864–14868. http://doi.org/10.1523/JNEUROSCI.3278-09.2009

Schenk, T., & McIntosh, R. D. (2010). Do we have independent visual streams for perception and action? *Cognitive Neuroscience*, *1*(1), 52–62. http://doi.org/10.1080/17588920903388950

Schiele, M. A., Reinhard, J., Reif, A., Domschke, K., Romanos, M., Deckert, J., & Pauli, P. (2016). Developmental aspects of fear: Comparing the acquisition and generalization of conditioned fear in children and adults. *Dev Psychobiol*, *58*(4), 471–81. Journal Article. http://doi.org/10.1002/dev.21393

Schmitz, A., & Grillon, C. (2012). Assessing fear and anxiety in humans using the threat of predictable and unpredictable aversive events (the npu-threat test). *Nature Protocols*, *7*(3), 527–532. Journal Article. http://doi.org/10.1038/nprot.2012.001

Schmukle, S. C. (2005). Unreliability of the dot probe task. *European Journal of Personality*, *19*(7), 595–605. Journal Article. http://doi.org/10.1002/per.554

Seligman, M., & Binik, Y. (1977). The safety signal hypothesis. In H. Davis & H. M. B. Hurwitz (Eds.), *Operant-pavlovian interactions* (pp. 165–187). Book Section, Hillsdale, New York.

Sevenster, D., Beckers, T., & Kindt, M. (2014). Fear conditioning of scr but not the startle reflex requires conscious discrimination of threat and safety. *Front Behav Neurosci*, *8*(32), 32. Journal Article. http://doi.org/10.3389/fnbeh.2014.00032

Sevenster, D., Visser, R. M., & D'Hooge, R. (2018). A translational perspective on neural circuits of fear extinction: Current promises and challenges. *Neurobiology of Learning and Memory*, *155*, 113–126.

Shackman, A. J., & Fox, A. S. (2016). Contributions of the central extended amygdala to fear and anxiety. *J Neurosci*, *36*(31), 8050–63. Journal Article. http://doi.org/10.1523/jneurosci.0982-16.2016

Shalev, L., Paz, R., & Avidan, G. (2018). Visual aversive learning compromises sensory discrimination. *J. Neurosci.*, *38*(11), 2766.

Sijbrandij, M., Engelhard, I. M., Lommen, M. J. J., Leer, A., & Baas, J. M. P. (2013). Impaired fear inhibition learning predicts the persistence of symptoms of posttraumatic stress disorder (ptsd). *Journal of Psychiatric Research*, *47*(12), 1991–1997.

Silva, B. A., Gross, C. T., & Gräff, J. (2016). The neural circuits of innate fear: Detection, integration, action, and memorization. *Learning & Memory*, *23*(10), 544–555.

Sjouwerman, R., Niehaus, J., Kuhn, M., & Lonsdorf, T. B. (2016). Don't startle me-interference of startle probe presentations and intermittent ratings with fear acquisition. *Psychophysiology*, *53*(12), 1889–1899. Journal Article. http://doi.org/10.1111/psyp.12761

Sokolov, E. N. (1963). Perception and the conditioned reflex.

Somerville, L. H., Wagner, D. D., Wig, G. S., Moran, J. M., Whalen, P. J., & Kelley, W. M. (2013). Interactions between transient and sustained neural signals support the generation and regulation of anxious emotion. *Cereb Cortex*, *23*(1), 49–60. Journal Article. http://doi.org/10.1093/cercor/bhr373

Somerville, L. H., Whalen, P. J., & Kelley, W. M. (2010). Human bed nucleus of the stria terminalis indexes hypervigilant threat monitoring. *Biol Psychiatry*, *68*(5), 416–24. Journal Article. http://doi.org/10.1016/j.biopsych.2010.04.002

Sperl, M. F. J., Panitz, C., Hermann, C., & Mueller, E. M. (2016). A pragmatic comparison of noise burst and electric shock unconditioned stimuli for fear conditioning research with

many trials. *Psychophysiology*, *53*(9), 1352–65. Journal Article. http://doi.org/10.1111/psyp.12677

Sperl, M. F. J., Panitz, C., Rosso, I. M., Dillon, D. G., Kumar, P., Hermann, A., … Mueller, E. M. (2019). Fear extinction recall modulates human frontomedial theta and amygdala activity. *Cerebral Cortex*, *29*(2), 701–715.

Spielberger, C. D., Gorsuch, R. L., & Lushene, R. E. (1970). *Manual for the state-trait anxiety inventory*. Book, Paolo Alto, CA: Consulting Psychologists Press.

Spitzer, R. L., Kroenke, K., Williams, J. B., & Lowe, B. (2006). A brief measure for assessing generalized anxiety disorder: The gad-7. *Arch Intern Med*, *166*(10), 1092–7. Journal Article. http://doi.org/10.1001/archinte.166.10.1092

Stegmann, Y., Ahrens, L., Pauli, P., Keil, A., & Wieser, M. J. (2020). Social aversive generalization learning sharpens the tuning of visuocortical neurons to facial identity cues. *Elife*, *9*, e55204.

Stegmann, Y., Reicherts, P., Andreatta, M., Pauli, P., & Wieser, M. J. (2019). The effect of trait anxiety on attentional mechanisms in combined context and cue conditioning and extinction learning. *Scientific Reports*, *9*(1), 8855. Journal Article. http://doi.org/10.1038/s41598-019-45239-3

Stegmann, Y., Schiele, M. A., Schümann, D., Lonsdorf, T. B., Zwanzger, P., Romanos, M., … Pauli, P. (2019). Individual differences in human fear generalization–pattern identification and implications for anxiety disorders. *Translational Psychiatry*, *9*(1), 307.

Stolarova, M., Keil, A., & Moratti, S. (2006). Modulation of the c1 visual event-related component by conditioned stimuli: Evidence for sensory plasticity in early affective perception. *Cereb Cortex*, *16*(6), 876–87. Journal Article. http://doi.org/10.1093/cercor/bhj031

Strauss, G. P., Allen, D. N., Jorgensen, M. L., & Cramer, S. L. (2005). Test-retest reliability of standard and emotional stroop tasks: An investigation of color-word and picture-word versions. *Assessment*, *12*(3), 330–337. http://doi.org/10.1177/1073191105276375

Stroop, J. R. (1935). Studies of interference in serial verbal reactions. *Journal of Experimental Psychology*, *18*(6), 643.

Sylvers, P., Lilienfeld, S. O., & LaPrairie, J. L. (2011). Differences between trait fear and trait anxiety: Implications for psychopathology. *Clin Psychol Rev*, *31*(1), 122–37. Journal Article. http://doi.org/10.1016/j.cpr.2010.08.004

Tamietto, M., Pullens, P., Gelder, B. de, Weiskrantz, L., & Goebel, R. (2012). Subcortical connections to human amygdala and changes following destruction of the visual cortex. *Current Biology*, *22*(15), 1449–1455. http://doi.org/10.1016/j.cub.2012.06.006

Taylor, S., Zvolensky, M. J., Cox, B. J., Deacon, B., Heimberg, R. G., Ledley, D. R., … Cardenas, S. J. (2007). Robust dimensions of anxiety sensitivity: Development and initial validation of the anxiety sensitivity index-3. *Psychol Assess*, *19*(2), 176–88. Journal Article. http://doi.org/10.1037/1040-3590.19.2.176

Thigpen, N. N., Bradley, M. M., & Keil, A. (2018a). Assessing the relationship between pupil diameter and visuocortical activity. *J Vis*, *18*(6), 7. Journal Article. http://doi.org/10.1167/18.6.7

Thigpen, N. N., Gruss, L. F., Garcia, S., Herring, D. R., & Keil, A. (2018b). What does the dot-probe task measure? A reverse correlation analysis of electrocortical activity. *Psychophysiology*, *55*(6), e13058. Journal Article. http://doi.org/10.1111/psyp.13058

Thigpen, N. N., Kappenman, E. S., & Keil, A. (2017). Assessing the internal consistency of the event-related potential: An example analysis. *Psychophysiology*, *54*(1), 123–138. Journal Article. http://doi.org/10.1111/psyp.12629

Tovote, P., Fadok, J. P., & Luthi, A. (2015). Neuronal circuits for fear and anxiety. *Nat Rev Neurosci*, *16*(6), 317–31. Journal Article. http://doi.org/10.1038/nrn3945

Van Essen, D. C., Anderson, C. H., & Felleman, D. J. (1992). Information processing in the primate visual system: An integrated systems perspective. *Science*, *255*(5043), 419–423. http://doi.org/10.1126/science.1734518

Vansteenwegen, D., Iberico, C., Vervliet, B., Marescau, V., & Hermans, D. (2008). Contextual fear induced by unpredictability in a human fear conditioning preparation is related to the chronic expectation of a threatening us. *Biological Psychology*, *77*(1), 39–46. Journal Article. http://doi.org/10.1016/j.biopsycho.2007.08.012

Vervliet, B., & Boddez, Y. (2020). Aversive stimulus pairings are an unnecessary and insufficient cause of pathological anxiety. *Biological Psychiatry*, *87*(10), 870–871.

Vervliet, B., & Raes, F. (2013). Criteria of validity in experimental psychopathology: Application to models of anxiety and depression. *Psychological Medicine*, *43*(11), 2241–2244. http://doi.org/10.1017/s0033291712002267

Vialatte, F. B., Maurice, M., Dauwels, J., & Cichocki, A. (2010). Steady-state visually evoked potentials: Focus on essential paradigms and future perspectives. *Prog Neurobiol*, *90*(4), 418–38. Journal Article. http://doi.org/10.1016/j.pneurobio.2009.11.005

Walker, D. L., & Davis, M. (1997). Double dissociation between the involvement of the bed nucleus of the stria terminalis and the central nucleus of the amygdala in startle increases produced by conditioned versus unconditioned fear. *J. Neurosci.*, *17*(23), 9375.

Wang, J., Clementz, B. A., & Keil, A. (2007). The neural correlates of feature-based selective attention when viewing spatially and temporally overlapping images. *Neuropsychologia*, *45*(7), 1393–1399. Journal Article. http://doi.org/10.1016/j.neuropsychologia.2006.10.019

Watson, J. B., & Rayner, R. (1920). Conditioned emotional reactions. *Journal of Experimental Psychology*, *3*(1), 1–14. Journal Article. http://doi.org/10.1037/h0069608

Weike, A. I., Hamm, A. O., Schupp, H. T., Runge, U., Schroeder, H. W., & Kessler, C. (2005). Fear conditioning following unilateral temporal lobectomy: Dissociation of conditioned startle potentiation and autonomic learning. *J Neurosci*, *25*(48), 11117–24. Journal Article. http://doi.org/10.1523/JNEUROSCI.2032-05.2005

Weike, A. I., Schupp, H. T., & Hamm, A. O. (2007). Fear acquisition requires awareness in trace but not delay conditioning. *Psychophysiology*, *44*(1), 170–80. Journal Article. http://doi.org/10.1111/j.1469-8986.2006.00469.x

Wells, A., & Matthews, G. (1994). *Attention and emotion: A clinical perspective*. Psychology Press.

Wells, A., & Matthews, G. (1996). Modelling cognition in emotional disorder: The s-ref model. *Behav Res Ther*, *34*(11-12), 881–8. Journal Article. http://doi.org/https://doi.org/10.1016/S0005-7967(96)00050-2

Wieser, M. J., Flaisch, T., & Pauli, P. (2014a). Raised middle-finger: Electrocortical correlates of social conditioning with nonverbal affective gestures. *PLoS One*, *9*(7), e102937. Journal Article. http://doi.org/10.1371/journal.pone.0102937

Wieser, M. J., & Keil, A. (2011). Temporal trade-off effects in sustained attention: Dynamics in visual cortex predict the target detection performance during distraction. *J Neurosci*, *31*(21), 7784–90. Journal Article. http://doi.org/10.1523/JNEUROSCI.5632-10.2011

Wieser, M. J., & Keil, A. (2014). Fearful faces heighten the cortical representation of contextual threat. *Neuroimage*, *86*, 317–25. Journal Article. http://doi.org/10.1016/j.neuroimage.2013.10.008

Wieser, M. J., & Keil, A. (2020). Attentional threat biases and their role in anxiety: A neurophysiological perspective. *International Journal of Psychophysiology*, *153*, 148–158.

Wieser, M. J., McTeague, L. M., & Keil, A. (2011). Sustained preferential processing of social threat cues: Bias without competition? *J Cogn Neurosci*, *23*(8), 1973–86. Journal Article. http://doi.org/10.1162/jocn.2010.21566

Wieser, M. J., Miskovic, V., & Keil, A. (2016a). Steady-state visual evoked potentials as a research tool in social affective neuroscience. *Psychophysiology*, *53*(12), 1763–1775. Journal Article. http://doi.org/10.1111/psyp.12768

Wieser, M. J., Miskovic, V., Rausch, S., & Keil, A. (2014b). Different time course of visuocortical signal changes to fear-conditioned faces with direct or averted gaze: A ssVEP study with single-trial analysis. *Neuropsychologia*, *62*, 101–10. Journal Article. http://doi.org/10.1016/j.neuropsychologia.2014.07.009

Wieser, M. J., Pauli, P., Weyers, P., Alpers, G. W., & Mühlberger, A. (2009). Fear of negative evaluation and the hypervigilance-avoidance hypothesis: An eye-tracking study. *Journal of Neural Transmission*, *116*(6), 717–723.

Wieser, M. J., Reicherts, P., Juravle, G., & Leupoldt, A. von. (2016b). Attention mechanisms during predictable and unpredictable threat - a steady-state visual evoked potential approach. *Neuroimage*, *139*, 167–175. Journal Article. http://doi.org/10.1016/j.neuroimage.2016.06.026

Withers, R. D., & Deane, F. P. (1995). Origins of common fears: Effects on severity, anxiety responses and memories of onset. *Behaviour Research and Therapy*, *33*(8), 903–915. http://doi.org/https://doi.org/10.1016/0005-7967(95)00044-X

Wittchen, H. U., Jacobi, F., Rehm, J., Gustavsson, A., Svensson, M., Jonsson, B., … Steinhausen, H. C. (2011). The size and burden of mental disorders and other disorders of the brain in europe 2010. *Eur Neuropsychopharmacol*, *21*(9), 655–79. Journal Article. http://doi.org/10.1016/j.euroneuro.2011.07.018

Yuan, M., Giménez-Fernández, T., Méndez-Bértolo, C., & Moratti, S. (2018). Ultrafast cortical gain adaptation in the human brain by trial-to-trial changes of associative strength in fear learning. *The Journal of Neuroscience*, *38*(38), 8262–8276. Journal Article. http://doi.org/10.1523/jneurosci.0977-18.2018

Zhaoping, L. (2019). A new framework for understanding vision from the perspective of the primary visual cortex. *Current Opinion in Neurobiology*, *58*, 1–10.

Publication list

Peer-reviewed journal articles

Stegmann, Y., Ahrens, L., Pauli, P., Keil, A., & Wieser, M. J. (2020). Social aversive generalization learning sharpens the tuning of visuocortical neurons to facial identity cues. *eLife*, 9, e55204, https://doi.org/10.7554/eLife.55204.

Stegmann, Y., Schiele, M.A., Schümann, D., Lonsdorf, T.B., Zwanzger, P., Romanos, M., Reif, A., Domschke, K., Deckert, J., Gamer, M., Pauli, P., 2019. Individual differences in human fear generalization—pattern identification and implications for anxiety disorders. *Translational Psychiatry*, 9(1), 307, https://doi.org/10.1038/s41398-019-0646-8.

Stegmann, Y., Reicherts, P., Andreatta, M., Pauli, P., & Wieser, M. J. (2019). The effect of trait anxiety on attentional mechanisms in combined context and cue conditioning and extinction learning. *Scientific Reports*, 9(1), 8855, https://doi.org/10.1038/s41598-019-45239-3.

Contributions to international conferences

Stegmann Y., Reicherts P., Andreatta M., Pauli P. and Wieser M. J. Attentional mechanisms in combined context and cue extinction learning using the NPU-threat test. Abstract for poster presentation, 2017, Annual Meeting of the Society for Psychophysiology, Vienna, October 2017.

Stegmann Y., Reicherts P., Andreatta, M., Pauli P. and Wieser M. J. "Attentional mechanisms in combined context and cue extinction learning using the NPU-threat test, 2017, World Association for Stress Related and Anxiety Disorders (WASAD), International Congress of the World Association for Stress Related and Anxiety Disorders, Würzburg, October 2017.

Stegmann Y., Reicherts P., Andreatta, M., Pauli P. and Wieser M. J. Attentional mechanisms in combined context and cue extinction learning using the NPU-threat test. Poster presentation, 2018, Annual Meeting of the European Meeting of Human Fear Conditioning, Cardiff, April, 2018.

Stegmann Y., Reicherts P., Pauli P. and Wieser M. J. Investigating attentional mechanisms during contextual anxiety and extinction learning using steady state VEPs, 2018, Jahrestagung Psychologie und Gehirn, Gießen, Juni 2018.

Stegmann Y., Reicherts P., Pauli P. and Wieser M. J. Investigating sustained attention in contextual anxiety using steady-state VEPs evoked by flickering video stimuli. Abstract for poster presentation, 2018, Annual Meeting of the Society for Psychophysiology, Quebec, October 2018.

Stegmann Y., Reicherts P., Pauli P. and Wieser M. J. Investigating attentional mechanisms in an orthogonal cue and context conditioning paradigm. Poster presentation, 2019, Annual Meeting of the European Meeting of Human Fear Conditioning, Würzburg, May, 2019.

Stegmann Y., Reicherts P., Pauli P. and Wieser M. J. Preliminary evidence for potentiated fear responses during contextual anxiety in a new orthogonal cue and context conditioning paradigm. Abstract for poster presentation, 2019, Jahrestagung Psychologie und Gehirn, Dresden, Juni 2019.

Stegmann Y., Reicherts P., Pauli P. and Wieser M. J. The Effect of sustained Anxiety on Phasic Fear Responses in a new orthogonal cue and context conditioning paradigm, 2019, Annual Meeting of the Society for Psychophysiology, Washington, D.C., September 2019.

Stegmann Y., Reicherts P., Pauli P. and Wieser M. J. The Effect of sustained Anxiety on Phasic Fear Responses in a new orthogonal cue and context conditioning paradigm, 2019, World Association for Stress Related and Anxiety Disorders (WASAD), International Congress of the World Association for Stress Related and Anxiety Disorders, Würzburg, October 2019.

Curriculum Vitae: Yannik Stegmann

Address:	Königsberger Straße 20, 97072 Würzburg, Germany
Date of Birth:	20.06.1992 in Aschaffenburg
Email:	yannikstegmann@gmail.com
Telephone:	+49 931 31-84357

Education

2016 - present:	Julius-Maximilian-University of Würzburg, Germany: Ph.D.-Student
2016 - present:	Julius-Maximilian-University of Würzburg, Germany: Psychological Psychotherapy
2014 - 2016:	Julius-Maximilian-University of Würzburg, Germany: Psychology (Master of Science, with distinction). Master-Thesis: "The influence of the experimenters' profession on psychologically induced placebo analgesia"
2011 - 2014:	Julius-Maximilian-University of Würzburg, Germany: Psychology (Bachelor of Science, with distinction)

Positions

2019 - present:	Clinical Psychologist at the University Clinic Würzburg, Germany
2016 - present:	Research scientist at the Department of Psychology I, Biological Psychology, Clinical Psychology and Psychotherapy, University of Würzburg, Germany
2012 - 2016:	Student Teaching Assistant for Statistics at the Department of Psychology III, Psychological Methods, Cognition and Applied Research, University of Würzburg, Germany

2012 - 2016:	Student Research Assistant at the Department of Psychology I, Biological Psychology, Clinical Psychology and Psychotherapy, University of Würzburg, Germany

Honors and Research stays

2019:	Poster Award - 11th European Meeting of Human Fear Conditioning
02 - 05/2019:	Research stay at the Center for the Study of Emotion and Attention, University of Florida, Gainesville, FL, USA
2018:	GSLS Travel Award, University of Würzburg
2018:	G.A. Lienert Award for the study of biopsychological methods
2013:	Deutschlandstipendium, University of Würzburg

Teaching

Seminar in Clinical Psychology (M.Sc.): Emotional disorders - Psychopathology and cognitive behavior therapy techniques

Seminar in Clinical Psychology (B.Sc.): Development and Maintenace of psychiatric disorders from a metacognitive perspective

Publications

Peer-reviewed journal articles

Stegmann, Y., Ahrens, L., Pauli, P., Keil, A., & Wieser, M. J. (2020). Social aversive generalization learning sharpens the tuning of visuocortical neurons to facial identity cues. *eLife*, 9, e55204, `https://doi.org/10.7554/eLife.55204`.

Stegmann, Y., Schiele, M.A., Schümann, D., Lonsdorf, T.B., Zwanzger, P., Romanos, M., Reif, A., Domschke, K., Deckert, J., Gamer, M., Pauli, P., 2019. Individual differences in human fear generalization—pattern identification and implications for anxiety disorders. *Translational Psychiatry*, 9(1), 307, `https://doi.org/10.1038/s41398-019-0646-8`.

Stegmann, Y., Reicherts, P., Andreatta, M., Pauli, P., & Wieser, M. J. (2019). The effect of trait anxiety on attentional mechanisms in combined context and cue conditioning and extinction learning. *Scientific Reports*, 9(1), 8855, `https://doi.org/10.1038/s41598-019-45239-3`.

Contributions to international conferences

Stegmann Y., Reicherts P., Andreatta M., Pauli P. and Wieser M. J. Attentional mechanisms in combined context and cue extinction learning using the NPU-threat test. Abstract for poster presentation, 2017, Annual Meeting of the Society for Psychophysiology, Vienna, October 2017.

Stegmann Y., Reicherts P., Andreatta, M., Pauli P. and Wieser M. J. "Attentional mechanisms in combined context and cue extinction learning using the NPU-threat test, 2017, World Association for Stress Related and Anxiety Disorders (WASAD), International Congress of the World Association for Stress Related and Anxiety Disorders, Würzburg, October 2017.

Stegmann Y., Reicherts P., Andreatta, M., Pauli P. and Wieser M. J. Attentional mechanisms in combined context and cue extinction learning using the NPU-threat test. Poster presentation, 2018, Annual Meeting of the European Meeting of Human Fear Conditioning, Cardiff, April, 2018.

Stegmann Y., Reicherts P., Pauli P. and Wieser M. J. Investigating attentional mechanisms during contextual anxiety and extinction learning using steady state VEPs, 2018, Jahrestagung Psychologie und Gehirn, Gießen, Juni 2018.

Stegmann Y., Reicherts P., Pauli P. and Wieser M. J. Investigating sustained attention in contextual anxiety using steady-state VEPs evoked by flickering video stimuli. Abstract for poster presentation, 2018, Annual Meeting of the Society for Psychophysiology, Quebec, October 2018.

Stegmann Y., Reicherts P., Pauli P. and Wieser M. J. Investigating attentional mechanisms in an orthogonal cue and context conditioning paradigm. Poster presentation, 2019, Annual Meeting of the European Meeting of Human Fear Conditioning, Würzburg, May, 2019.

Stegmann Y., Reicherts P., Pauli P. and Wieser M. J. Preliminary evidence for potentiated fear responses during contextual anxiety in a new orthogonal cue and context conditioning paradigm. Abstract for poster presentation, 2019, Jahrestagung Psychologie und Gehirn, Dresden, Juni 2019.

Stegmann Y., Reicherts P., Pauli P. and Wieser M. J. The Effect of sustained Anxiety on Phasic Fear Responses in a new orthogonal cue and context conditioning paradigm, 2019, Annual Meeting of the Society for Psychophysiology, Washington, D.C., September 2019.

Stegmann Y., Reicherts P., Pauli P. and Wieser M. J. The Effect of sustained Anxiety on Phasic Fear Responses in a new orthogonal cue and context conditioning paradigm, 2019, World Association for Stress Related and Anxiety Disorders (WASAD), International Congress of the World Association for Stress Related and Anxiety Disorders, Würzburg, October 2019.

_____ _____

Place, Date Signature

Affidavit

I hereby confirm that my thesis entitled “Electrocortical mechanisms of sustained attention during acquisition and extinction of conditioned fear and anxiety” 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