Electrophysiological Signatures of Fear Conditioning:

From Methodological Considerations to Catecholaminergic Mechanisms and Translational Perspectives

Elektrophysiologische Korrelate von Furchtkonditionierung:

Von methodischen Überlegungen zu katecholaminergen Mechanismen und translationalen Perspektiven

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Matthias F. J. Sperl (M. Sc.)

aus Oberviechtach

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Zweitgutachter: Prof. Dr. Rudolf Stark (Justus-Liebig-Universität Gießen)
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Abstract

Fear conditioning describes a learning mechanism during which a specific stimulus gets associated with an aversive event (i.e., an unconditioned stimulus; US). Thereby, this initially neutral or arbitrary stimulus becomes a so-called “conditioned” stimulus (CS), which elicits a conditioned threat response. Fear extinction refers to the decrease in conditioned threat responses as soon as the CS is repeatedly presented in the absence of the US. While fear conditioning is an important learning model for understanding the etiology and maintenance of anxiety and fear-related disorders, extinction learning is considered to reflect the most important learning process of exposure therapy. Neurophysiological signatures of fear conditioning have been widely studied in rodents, leading to the development of groundbreaking neurobiological models, including brain regions such as the amygdala, insula, and prefrontal areas. These models aim to explain neural mechanisms of threat processing, with the ultimate goal to improve treatment strategies for pathological fear. Recording intracranial electrical activity of single units in animals offers the opportunity to uncover neural processes involved in threat processing with excellent spatial and temporal resolution. A large body of functional magnetic resonance imaging (fMRI) studies have helped to translate this knowledge about the anatomy of fear conditioning into the human realm. fMRI is an imaging technique with a high spatial resolution that is well suited to study slower brain processes. However, the temporal resolution of fMRI is relatively poor. By contrast, electroencephalography (EEG) is a neuroscientific method to capture fast and transient cortical processes. While EEG offers promising opportunities to unravel the speed of neural threat processing, it also provides the possibility to study oscillatory brain activity (e.g., prefrontal theta oscillations). The present thesis contains six research manuscripts, describing fear conditioning studies that mainly applied **EEG methods** in combination with **other central (fMRI) and peripheral (skin conductance, heart rate, and fear-potentiated startle) measures**. A special focus of this thesis lies in **methodological considerations** for EEG fear conditioning research. Furthermore, **catecholaminergic mechanisms** are studied, with the ultimate goal of opening up new **translational perspectives**.

The EEG method requires averaging across a massive number of trials to achieve an adequate signal-to-noise ratio. This endeavor can be problematic, given that aversive responses to the US may habituate over time. In **manuscript 1** (which has been **published in *Psychophysiology***), a between-subjects design was used to **compare two commonly used US types (electric shock and white noise burst)** in a 260-trial fear conditioning and extinction paradigm. The paradigm has been optimized for EEG recordings and includes many trials to ensure a sufficient signal-to-noise ratio. Affective CS ratings and peripheral physiological measures pointed toward more reliable, extinction-resistant, and stable conditioning with white noise bursts. At the same time, the aversive properties of the electric shocks seemed to diminish across acquisition trials.

The first manuscript demonstrates that selecting appropriate US types is crucial for the development of a robust conditioned response. Besides this methodological aspect, the nature of the US is also highly relevant for the validity of fear conditioning paradigms as etiological models for

pathological fear. It has often been criticized that fear conditioning (a learning process that involves the pairing of initially neutral or arbitrary stimuli with an aversive event like an electric shock) may not be an appropriate laboratory model for anxiety disorders. In particular, many patients are not able to recall physical CS-US pairings (e.g., traumatic events) in their past. **Manuscript 2** (which has been **published in *Psychological Science***) includes two studies demonstrating that **aversive mental images (e.g., the imagery of receiving an electric shock), which are paired with an initially neutral CS, can cause *de novo* fear learning**. Remarkably, these studies demonstrate threat learning in the absence of any external aversive stimulation. Importantly, manuscript 2 emphasizes the etiological relevance of fear conditioning models for understanding pathological fear. Thus, these results lay the ground for the clinical relevance of all further EEG findings of this thesis: The results described in manuscript 2 provide empirical evidence that aversive imagery may be sufficient for successful fear conditioning. These findings highlight the vital role of fear conditioning research even for those patients who cannot report the experience of an external aversive event in their etiological history.

Previous EEG fear conditioning studies have helped to elucidate critical perceptual and attentional brain processes, but how fear memory traces evolve and change over time has been widely ignored. As explained above (see manuscript 1), aggregations across high numbers of trials are required due to the low signal-to-noise ratio of EEG. However, this approach will wash out any transient neurophysiological processes, which are related to learning and prone to habituation. **Manuscript 3** (which has been **published in *NeuroImage***) describes the **development and validation of a new sequential-set fear conditioning paradigm**. This design comprises three successive conditioning and extinction stages, each with a novel CS+/CS- set (the CS+ is a CS that gets paired with an aversive US during acquisition; the CS- is not paired with a US). Averaging across these three CS+/CS- sets triples the relative amount of trials that tap into more transient brain processes and allowed us to unravel the **learning curves of neural responses**. This approach uncovers how short-latency (33–60 ms post-CS), mid-latency (108–200 ms), and long-latency (468–820 ms) EEG responses increase and decrease during fear conditioning and extinction, respectively.

Research in animals suggests that hyperconsolidation of aversive memories in the etiology of fear-related disorders may be associated with an elevated release or abnormal regulation of catecholaminergic neurotransmitters (especially noradrenaline and dopamine). Conversely, catecholaminergic drugs seem to be promising for facilitating the efficacy of exposure therapies. In **manuscript 4** (which has been **submitted to *Neuropsychopharmacology***), participants received the noradrenergic substance yohimbine, the dopaminergic substance sulpiride, or a placebo pill between fear conditioning and extinction. Peripheral (fear-conditioned bradycardia) and central (N170 and late positive potential EEG responses) physiological measures indicated that **yohimbine treatment enhanced fear recall on the following day**. These data suggest that noradrenaline potentiates cardiac and neural signatures of fear memory consolidation. Noradrenaline-related

hyperconsolidation of aversive associations may be a key neurophysiological mechanism in the etiology of anxiety and fear-related disorders.

The crucial role of catecholamines in fear conditioning and extinction is further corroborated by **manuscript 5** (which has been **published in *Neurobiology of Learning and Memory***). In this study, participants were screened for the catechol-*O*-methyltransferase (*COMT*) Val158Met genotype and underwent a two-day fear conditioning and extinction procedure. Previous research suggests an association between the Val/Val genotype and relatively lower catecholamine levels (especially prefrontal dopamine levels). In the data described in manuscript 5, **Val homozygotes showed the most adaptive response pattern during a recall test 24 hours after fear conditioning and extinction** – as indicated by elevated conditioned responses for nonextinguished threat cues, but reduced conditioned responses for extinguished threat cues. Similarly to manuscript 4, heart rate (fear-conditioned bradycardia) and EEG (late positive potential amplitudes) data revealed converging results in this study. This knowledge underlines the critical role of catecholamines in fear processing. In light of stratified therapeutic strategies, these findings may help to develop innovative and individually tailored interventions.

In the previous manuscripts, EEG was used to study how event-related potentials are modulated by fear conditioning and catecholaminergic transmission. However, in addition to these rather traditional EEG methods, frequency-based EEG analyses allow one to probe oscillatory brain activity. Neural oscillations seem to be critical for communication between brain areas (e.g., between the amygdala and prefrontal regions). In **manuscript 6** (which has been **published in *Cerebral Cortex***), EEG and fMRI were recorded simultaneously during the recall of conditioned and extinguished fear, 24 hours after conditioning and extinction. **Frequency-based EEG** measures of fear and extinction recall, as indicated by **frontomedial theta power, explained 60% of the variance for the analogous effect in the right amygdala (fMRI)**. Combining both neuroscientific methods highlights the interplay between amygdala and prefrontal theta activity. These findings provide insight into neural circuits consistently linked with top-down amygdala modulation in rodents.

Taken together, the present thesis addresses several methodological challenges for neuroscientific (in particular, EEG) fear conditioning research (e.g., appropriate US types and experimental designs, signal-to-noise ratio, simultaneous EEG-fMRI). Furthermore, this thesis gives critical insight into catecholaminergic (noradrenaline and dopamine) mechanisms. A variety of neuroscientific methods (e.g., EEG, fMRI, peripheral physiology, pharmacological manipulation, genetic associations) have been combined, an approach that allowed us (a) to translate knowledge from animal studies to human research, and (b) to stimulate novel clinical directions.

Zusammenfassung (Abstract in German)

Furchtkonditionierung beschreibt einen Lernmechanismus, bei dem ein spezifischer Reiz mit einem aversiven Ereignis (d.h. einem unkonditionierten Reiz; US) assoziiert wird. Dadurch wird dieser zunächst neutrale oder beliebig ausgewählte Reiz zu einem sogenannten „konditionierten" Reiz (CS), der eine konditionierte Bedrohungs-Reaktion hervorruft. Unter Furchtextinktion versteht man die Abnahme der konditionierten Bedrohungs-Reaktion, sobald der CS wiederholt in Abwesenheit des US präsentiert wird. Während die Furchtkonditionierung ein wichtiges Lernmodell für das Verständnis der Ätiologie und Aufrechterhaltung von Angststörungen und Furcht-assoziierten Störungen darstellt, wird das Extinktionslernen als der wichtigste Lernprozess bei der Expositionstherapie angesehen. Neurophysiologische Korrelate der Furchtkonditionierung wurden umfassend in Nagetieren untersucht, was zur Entwicklung wegweisender neurobiologischer Modelle führte, welche Gehirnregionen wie die Amygdala, die Insula und präfrontale Areale beinhalten. Diese Modelle zielen darauf ab, neuronale Mechanismen der Bedrohungs-Verarbeitung zu erklären, mit dem letztendlichen Ziel, Behandlungs-Strategien für pathologische Furcht zu verbessern. Die Aufzeichnung der intrakraniellen elektrischen Aktivität einzelner Nervenzellen bietet im Tiermodell die Möglichkeit, neuronale Prozesse, die an der Bedrohungs-Verarbeitung beteiligt sind, mit hervorragender räumlicher und zeitlicher Auflösung aufzudecken. Eine große Anzahl von Studien mittels funktioneller Magnetresonanztomographie (fMRT) hat dazu beigetragen, dieses Wissen über anatomische Grundlagen der Furchtkonditionierung auf den Menschen zu übertragen. fMRT ist ein bildgebendes Verfahren mit hoher räumlicher Auflösung, das sich gut für die Untersuchung langsamerer Gehirnprozesse eignet. Allerdings ist die zeitliche Auflösung der fMRT relativ niedrig. Im Gegensatz dazu ist die Elektroenzephalographie (EEG) eine neurowissenschaftliche Methode, die sich hervorragend zur Erfassung schneller und transienter kortikaler Prozesse eignet. Während die EEG-Methodik vielversprechende Möglichkeiten eröffnet, die Geschwindigkeit neuronaler Bedrohungs-Verarbeitung zu entschlüsseln, bietet sie auch die Möglichkeit, oszillatorische Hirnaktivität (z. B. präfrontale Theta-Oszillationen) zu untersuchen. Die vorliegende Dissertation enthält sechs Forschungs-Manuskripte und beschreibt Studien zur Furchtkonditionierung, bei denen hauptsächlich **EEG-Methoden** in Kombination mit **anderen zentral- (fMRT) und peripher-physiologischen Methoden (elektrodermale Aktivität, Herzfrequenz und Modulation des Schreckreflexes)** eingesetzt wurden. Ein besonderer Schwerpunkt dieser Arbeit liegt auf **methodischen Überlegungen** zur Furchtkonditionierungs-Forschung mit EEG. Darüber hinaus werden **katecholaminerge Mechanismen** untersucht, mit dem langfristigen Ziel, neue **translationale Perspektiven** zu eröffnen.

Die EEG-Methode erfordert ein Mitteln über eine sehr große Anzahl an Lerndurchgängen, um ein angemessenes Signal-Rausch-Verhältnis zu erreichen. Dieses Vorgehen kann problematisch sein, da aversive Reaktionen auf den US mit der Zeit habituieren können. In **Manuskript 1** (**publiziert in *Psychophysiology***) wurde ein Zwischensubjekt-Design verwendet, um **zwei häufig verwendete US-Typen (einen elektrischen Reiz und weißes Rauschen)** in einem Furchtkonditionierungs- und

Furchtextinktions-Paradigma mit 260 Durchgängen **zu vergleichen**. Das Paradigma wurde für EEG-Aufzeichnungen optimiert und beinhaltet deshalb viele Durchgänge, um ein ausreichendes Signal-Rausch-Verhältnis zu gewährleisten. Affektive Bewertungen der CS und peripher-physiologische Messungen wiesen auf eine stärkere, extinktionsresistentere und stabilere Konditionierung mit weißem Rauschen hin. Gleichzeitig schienen die aversiven Eigenschaften der elektrischen Reize über die Akquisitions-Durchgänge hinweg abzunehmen.

Das erste Manuskript zeigt, dass die Auswahl geeigneter US-Typen entscheidend für die Entstehung einer robusten konditionierten Reaktion ist. Neben diesem methodischen Aspekt ist die Art des US auch von hoher Relevanz für die Validität von Furchtkonditionierungsparadigmen als ätiologische Modelle für pathologische Furcht. Es wurde oft kritisiert, dass Furchtkonditionierung (ein Lernprozess, der die Paarung von anfänglich neutralen oder beliebigen Reizen mit einem aversiven Ereignis, wie z. B. einem elektrischen Reiz, beinhaltet) möglicherweise kein geeignetes Labormodell für Angststörungen ist. Insbesondere sind viele Menschen mit Angststörungen nicht in der Lage, sich an physische CS-US-Paarungen (z. B. traumatische Ereignisse) in ihrer Vergangenheit zu erinnern. **Manuskript 2** (**publiziert in *Psychological Science***) enthält zwei Studien, die zeigen, dass **aversive Imaginationen (z. B. die Vorstellung, einen elektrischen Reiz zu erhalten), die mit einem ursprünglich neutralen CS gepaart werden, zu *de novo* Furchtlernen führen können**. Bemerkenswert ist, dass diese Studien das Lernen von Bedrohung in Abwesenheit jeglicher externer aversiver Stimulation aufzeigen. Manuskript 2 hebt die ätiologische Relevanz von Furchtkonditionierungs-Modellen für das Verständnis pathologischer Furcht hervor. Damit legen diese Ergebnisse den Grundstein für die klinische Relevanz aller weiteren EEG-Befunde dieser Dissertation: Die in Manuskript 2 beschriebenen Ergebnisse liefern empirische Evidenz dafür, dass eine aversive Imagination für eine erfolgreiche Furchtkonditionierung ausreichen kann. Diese Befunde unterstreichen die wichtige Rolle der Furchtkonditionierungs-Forschung auch für jene Personen mit Angststörungen, die in ihrer ätiologischen Vorgeschichte keine Erfahrung eines externen aversiven Ereignisses berichten können.

Frühere Furchtkonditionierungs-Studien mit EEG haben maßgeblich zum Verständnis relevanter Wahrnehmungs- und Aufmerksamkeits-bezogener Prozesse im Gehirn beigetragen; es wurde jedoch weitgehend ignoriert, wie sich Furchtgedächtnis-Spuren entwickeln und über die Zeit verändern. Wie oben erläutert (siehe Manuskript 1), erfordert die EEG-Methodik aufgrund des niedrigen Signal-Rausch-Verhältnisses Aggregationen über eine große Anzahl von Lerndurchgängen. Dieser Ansatz verhindert jedoch die Erfassung von transienten neurophysiologischen Prozessen, die mit dem Lernen zusammenhängen und anfällig für Habituation sind. **Manuskript 3** (**publiziert in *NeuroImage***) beschreibt die **Entwicklung und Validierung eines neuen „sequential-set“ Furcht-konditionierungs-Paradigmas**. Dieses experimentelle Paradigma umfasst drei aufeinanderfolgende Konditionierungs- und Extinktions-Phasen, jede mit einem neuen CS+/CS- Stimulus-„Set“ (der CS+ ist ein CS, der während der Furcht-Akquisition mit einem unangenehmen US gepaart wird; der CS- wird nicht mit einem US gepaart). Das Mitteln über diese drei CS+/CS- „Sets“ verdreifacht die relative

Anzahl von Durchgängen, die eher transiente Gehirnprozesse abbilden, und ermöglicht es so, die **Lernkurven der neuronalen Reaktionen** zu entschlüsseln. Dieser Ansatz veranschaulicht, wie EEG-Reaktionen mit kurzer (33–60 ms post-CS), mittlerer (108–200 ms) und langer Latenz (468–820 ms) während der Furchtkonditionierung und Furchtextinktion zunehmen bzw. abnehmen.

Forschungsarbeiten an Tiermodellen weisen darauf hin, dass in der Ätiologie Furcht-assoziierter Störungen die Hyperkonsolidierung aversiver Erinnerungen mit einer erhöhten Ausschüttung oder veränderten Regulation katecholaminerger Neurotransmitter (insbesondere Noradrenalin und Dopamin) verbunden sein könnte. Umgekehrt erscheint es vielversprechend, dass katecholaminerge Substanzen die Wirksamkeit von Expositionstherapien unterstützen könnten. In **Manuskript 4** (**eingereicht bei *Neuropsychopharmacology***) erhielten die Versuchspersonen zwischen der Furchtkonditionierung und Furchtextinktion die noradrenerge Substanz Yohimbin, die dopaminerge Substanz Sulpirid oder ein Placebo. Peripher-physiologische (Herzfrequenz: Furcht-konditionierte Bradykardie) und zentral-physiologische (EEG: ereigniskorrelierte Potentiale, N170 und LPP) Methoden zeigten, dass die **Yohimbin-Gabe den Furcht-Abruf am nächsten Tag erhöhte**. Diese Daten deuten darauf hin, dass Noradrenalin kardiale (Herzfrequenz) und neuronale (EEG) Indikatoren der Furchtgedächtnis-Konsolidierung potenziert. Eine Noradrenalin-vermittelte Hyperkonsolidierung aversiver Assoziationen könnte ein wichtiger neurophysiologischer Mechanismus in der Ätiologie von pathologischer Furcht sein.

Die bedeutsame Rolle von Katecholaminen bei der Furchtkonditionierung und -extinktion wird durch **Manuskript 5** (**publiziert in *Neurobiology of Learning and Memory***) weiter untermauert. In dieser Studie wurden die Versuchspersonen zunächst hinsichtlich des Catechol-*O*-Methyltransferase (*COMT*) Val158Met Polymorphismus genotypisiert, bevor sie an einem zweitägigen Furchtkonditionierungs- und Fuchtextinktions-Paradigma teilnahmen. Frühere Untersuchungen deuten auf einen Zusammenhang zwischen dem Val/Val-Genotyp und relativ niedrigeren Katecholamin-Spiegeln hin (insbesondere präfrontale Dopamin-Spiegel). In den in Manuskript 5 beschriebenen Daten zeigten **Personen mit dem Val/Val-Genotyp das adaptivste Reaktionsmuster während eines Abruf-Tests 24 Stunden nach der Konditionierung und Extinktion** – abgebildet durch erhöhte konditionierte Reaktionen für nicht-extingierte Bedrohungs-Reize, aber reduzierte konditionierte Reaktionen für extingierte Bedrohungs-Reize. Ähnlich wie in Manuskript 4 waren die Ergebnisse in Bezug auf Herzfrequenz (Furcht-konditionierte Bradykardie) und EEG (LPP-Amplituden) in dieser Studie konvergierend. Diese Erkenntnisse unterstreichen die wichtige Rolle von Katecholaminen bei der Furcht-Verarbeitung. Im Hinblick auf stratifizierte therapeutische Ansätze können diese Erkenntnisse helfen, innovative und individuell zugeschnittene Interventionen zu entwickeln.

In den vorangegangenen Manuskripten wurde EEG verwendet, um zu untersuchen, wie ereigniskorrelierte Potentiale durch Furchtkonditionierung und katecholaminerge Übertragung moduliert werden. Zusätzlich zu diesen eher traditionellen EEG-Methoden ermöglichen frequenzbasierte EEG-Analysen die Untersuchung oszillatorischer Gehirnaktivität. Neuronale

Oszillationen scheinen entscheidend für die Kommunikation zwischen Gehirnarealen zu sein (z. B. zwischen der Amygdala und präfrontalen Regionen). In **Manuskript 6** (**publiziert in *Cerebral Cortex***) wurden EEG und fMRT gleichzeitig während des Abrufs von konditionierter und extingierter Furcht, 24 Stunden nach Konditionierung und Extinktion, erhoben. **Frequenzbasierte EEG-Maße** des Furcht- und Extinktionsabrufs, operationalisiert durch **frontomediale Theta-Leistungsdichte, erklärten 60% der Varianz für den analogen Effekt in der rechten Amygdala (fMRT)**. Die Kombination dieser beiden neurowissenschaftlichen Methoden hebt das Zusammenspiel zwischen der Amygdala und präfrontaler Theta-Aktivität hervor. Diese Befunde geben Einblick in neuronale Schaltkreise, welche bereits in Tiermodellen (Nagetiere) konsistent mit einer „top-down“ Modulation der Amygdala verknüpft wurden.

Insgesamt adressiert die vorliegende Dissertation mehrere methodische Herausforderungen für die neurowissenschaftliche (insbesondere EEG) Furchtkonditionierungs-Forschung (z. B. geeignete US-Typen und experimentelle Paradigmen, Signal-Rausch-Verhältnis, simultane EEG-fMRT). Darüber hinaus gibt diese Arbeit wichtige Einblicke in katecholaminerge (Noradrenalin und Dopamin) Mechanismen. Eine Vielzahl neurowissenschaftlicher Methoden (z. B. EEG, fMRT, peripher-physiologische Maße, pharmakologische Manipulation, genetische Assoziationen) wurde kombiniert. Dieser Ansatz ermöglichte es uns, (a) Erkenntnisse aus Tiermodellen in die Humanforschung zu übertragen und (b) neue klinische Perspektiven aufzuzeigen.

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Content

Abbreviations

ANCOVA	analysis of covariance
ANOVA	analysis of variance
BOLD	blood oxygenation level dependent contrast
AMC	anterior midcingulate cortex
BLA	basolateral nucleus of the amygdala
CEA	central nucleus of the amygdala
COMT, *COMT*	catechol-*O*-methyltransferase Note: This abbreviation is italicized (*COMT*) whenever it reflects the gene.
COMT Val158Met	the Val/Met polymorphism on codon 158 of the *COMT* gene
CS	conditioned stimulus
CS+	conditioned stimulus (CS) paired with an aversive unconditioned stimulus (US) during acquisition training
CS-	conditioned stimulus (CS) *not* paired with an unconditioned stimulus (US) during acquisition training
DA	dopamine
dACC	dorsal anterior cingulate cortex
FER	"fear and extinction recall" score, calculated as: FER = (CS+N minus CS-N) minus (CS+E minus CS-E)
IL	infralimbic cortex
LA	lateral nucleus of the amygdala
LPP	late positive potential
MRI	magnetic resonance imaging
PAG	periaqueductal gray
PL	prelimbic cortex
PTSD	post-traumatic stress disorder
CS+E	"extinguished" CS+ (CS+ presented during extinction training)
CS-E	"extinguished" CS- (CS- presented during extinction training)
CS+N	"*non*extinguished" CS+ (CS+ *not* presented during extinction training)
CS-N	"*non*extinguished" CS- (CS- *not* presented during extinction training)
EEG	electroencephalography
ERP	event-related potential
fMRI	functional magnetic resonance imaging
IBI	interbeat interval (heart period)
ITI	intertrial interval (defined as CS offset to CS onset)
MEG	magnetoencephalography
NE	noradrenaline/norepinephrine
sLORETA	standardized low resolution brain electromagnetic tomography
SCR	skin conductance response
US	unconditioned stimulus
vmPFC	ventromedial prefrontal cortex

“Trends come and go in psychology. Topics that are hot today will be cold in 10 or even 5 years, but some parts of psychology continue to build systematic and important data bases and theories. The study of sensory mechanisms is one example. **I think that the study of the associative mechanisms underlying Pavlovian conditioning is another**. These fields are enduring and systematic, but I hope it is now obvious that they are also changing and exciting.”

Robert A. Rescorla, “Pavlovian Conditioning – It’s Not What You Think It Is”;
published in 1988 in the journal *American Psychologist*.

(bold emphasis added by Matthias F. J. Sperl)

1 Introduction

All human beings have experienced "fear" in their lives. "Fear" is a basic emotion (Ekman, 1992), which is elicited by danger or life-threatening emergencies and accompanied by an activation of the autonomic nervous system (LaBar, 2018; LeDoux & Hofmann, 2018). The experience of "fear" often terminates into "fight-or-flight" behavior to promote survival (Davis & Lang, 2003; LaBar, 2018). After a long debate regarding precise terminology (Adolphs, 2013; Fanselow & Pennington, 2017; LeDoux, 2014; LeDoux & Daw, 2018; LeDoux & Hofmann, 2018; Mobbs, 2018), the term "fear" has recently been defined as a "*mental state* … [describing] … feelings that occur when the source of harm, the threat, is either immediate or imminent" (LeDoux & Pine, 2016). Specifically, to disentangle and to clarify threat-related processes, LeDoux and colleagues (LeDoux, 2017; LeDoux & Daw, 2018; LeDoux & Pine, 2016) have introduced a so-called "two-system" framework (see Figure 1). On the one hand (green boxes in Figure 1), an immediately present threat activates cortical brain areas (including the prefrontal cortex), which leads to the conscious feeling of fear (i.e., self-reported "fear"). On the other hand (orange boxes in Figure 1), the activation of subcortical brain areas ("defensive" circuits, including the amygdala) generates peripheral physiological adjustments and behavioral responses to threat. According to this model, physiological and behavioral changes can be dissociated from the subjective state of "fear". However, it is important to emphasize the interaction between both brain circuits (blue arrows in Figure 1). Physiological and behavioral adjustments can thus be associated with changes in the subjective state of an individual (Pine & LeDoux, 2017).

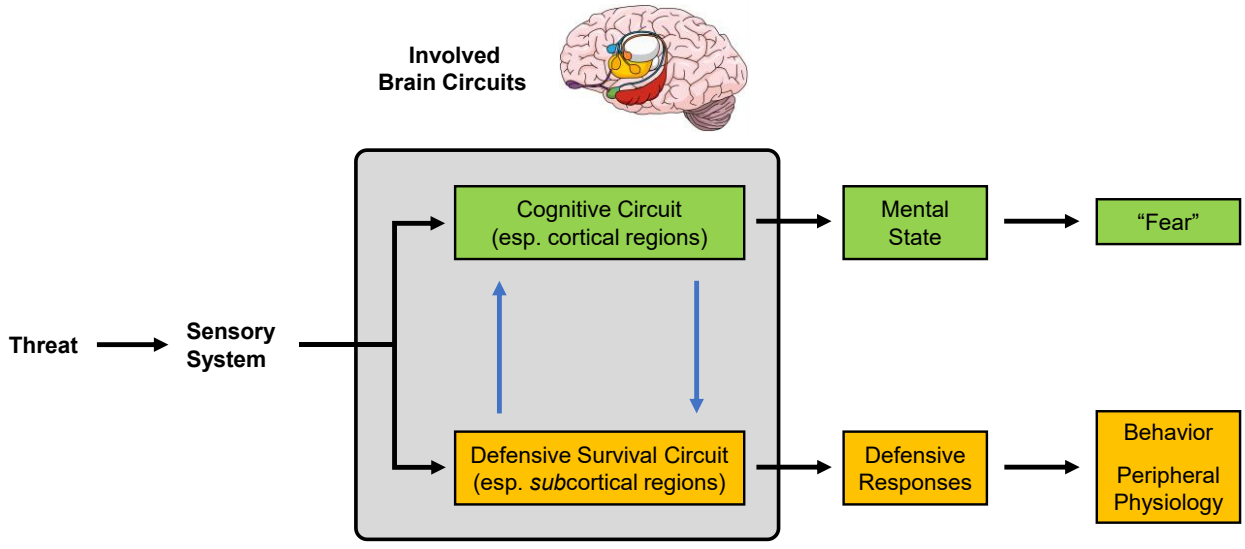


Figure 1. Threat activates several brain areas. The "two-system" view of fear (e.g., LeDoux & Pine, 2016) distinguishes two circuits: While the conscious experience of "fear" emerges from a "cognitive" circuit, a "defensive survival" circuit initiates defensive behavior and related peripheral physiological responses.

1.1 100 Years of Fear Conditioning: A Successful Translational Model for Psychology

As outlined above, the mental state of "fear" is part of an alarm reaction to imminent danger. Elevated fear responses and heightened attention toward threats facilitate survival (LeDoux & Daw, 2018). However, exaggerated or abnormally regulated fear can be a core pathophysiological mechanism in the etiology of many fear- and stressor-related disorders (Maddox et al., 2019; Ressler, 2020). Anxiety disorders (Craske et al., 2017), obsessive-compulsive disorder (Stein et al., 2019), and post-traumatic stress disorder (PTSD; Yehuda et al., 2015) are highly prevalent conditions. These disorders cause severe distress in a patient's life (e.g., personal relationships, job performance) and are a leading cause of disability in many societies (Kessler et al., 2007, 2009; Wittchen et al., 2011). But how is it possible that certain stimuli or situations (e.g., confrontation with a dog), which are not or considerably less threatening for most healthy people, can cause such an excessive and unreasonable subjective state of fear in other individuals (e.g., patients with dog phobia)? In this context, what is the functional relevance of defensive behavioral responses (e.g., avoidance of dogs) and corresponding peripheral physiological reactions (e.g., increase in heart rate, sweating)? Why do these reactions, which are out of proportion to the actual threat posed, even spread to other cues or situations (e.g., associated locations, semantically related stimuli) and impair normal functioning in everyday life (Dymond et al., 2015)? Why are anxiety and related disorders so prevalent? And – of particular relevance for clinical fear – is it possible to "unlearn" or "erase" fear?

To find answers to these questions, fear conditioning paradigms have been widely used in animal (Tovote et al., 2015) and human (Fullana et al., 2020) research. Classical (also called *Pavlovian*; Pavlov, 1906) conditioning describes a learning mechanism through which initially arbitrary (or even neutral) stimuli become associated with biologically relevant stimuli (LeDoux & Daw, 2018). As outlined before, to ensure survival, humans react with genetically hardwired defensive behavior when faced with an *immediate* threat (LeDoux & Pine, 2016). As illustrated in Figure 2, fear – together with associated changes in behavior and peripheral physiology – can also be *learned* through Pavlovian conditioning (Kim & Jung, 2018). Such "conditioned" responses are then expressed in anticipation of *imminent* danger that is *very close* in time. For example, after a child has been bitten by a dog, this child will experience fear upon further encounters with dogs. Humans and animals are biologically predisposed to learn associations between danger (e.g., a predatory attack) and certain stimuli (e.g., specific elements of a predator), due to their evolutionary preparedness (Öhman & Mineka, 2001). *Conditioned* fear (see Figure 2) can be further distinguished from *anxiety*, which is more future-oriented and describes a rather sustained

state of worry about potential danger (Barlow, 2000; Carleton, 2016). States of anxiety are not necessarily elicited by a specific stimulus (Corr, 2009; Corr & Krupić, 2020; McNaughton & Corr, 2004, 2018; Perusini & Fanselow, 2015). Anxiety is considered to be much vaguer and more diffuse ("I am worried about what *might* happen … Watch out, be very careful!") than fear ("I *am* in danger! Get me out of here!"). As explained in the next section, conditioned fear refers to associations between stimuli, which allows a precise prediction of danger. Such a reliable and valid prediction of future threat (which would lead to adaptive "fight-or-flight" behavior) is typically not (or at least less) possible during the longer-lasting state of anxiety (Schmitz & Grillon, 2012).

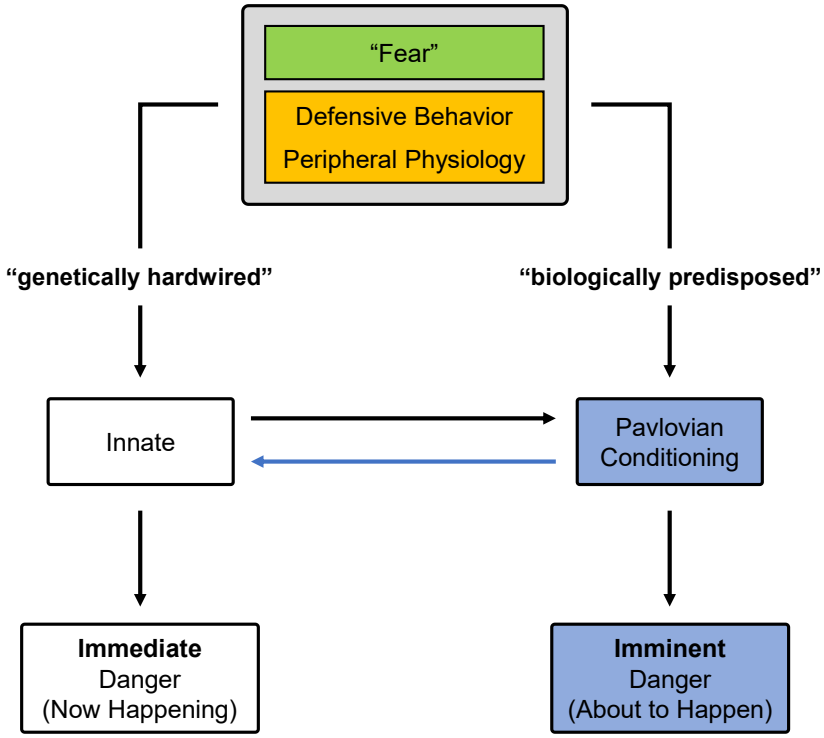


Figure 2. The "natural structure" of fear, as suggested by Kim and Jung (2018). Fear (and related behavioral/physiological changes) can functionally be divided into (left, white boxes) "genetically hardwired" innate responses to immediate danger and (right, blue boxes) "biologically predisposed" learned responses. Pavlovian conditioning is a core learning mechanism for learned ("conditioned") fear. Conditioned fear occurs in anticipation of imminent danger, which will happen very soon.

Fear conditioning describes how associations are learned between threatening stimulation and nonthreatening cues in the environment (Fanselow & Sterlace, 2014). The learning procedures behind fear conditioning can be best explained by its method, as this allows one to maintain strict control regarding the presentation of involved stimuli (Gottlieb & Begej, 2014). In typical fear conditioning experiments, an innocuous cue (the *conditioned stimulus*; CS) is paired with an

unconditioned stimulus (US). The US automatically and reliably activates a "defensive" system. The US generates an *unconditioned response*, which is considered to be innate and does not require prior learning. After pairing with the US, the CS becomes a reliable predictor of the US. Thereby, the CS also elicits a defensive response, the so-called *conditioned response*. In contrast to the unconditioned response, this conditioned response is *learned*. As an example, after a dog bite (US), dogs can become CSs and elicit conditioned fear. It is important to note that conditioned responses can be similar to the unconditioned response; however, they can also be qualitatively different, a factor that is often neglected in fear conditioning research (see chapter 3.4 in the discussion section). The terms *conditioned stimulus* (CS) and *unconditioned stimulus* (US) have been frequently used in current research to indicate a connection between stimuli (Lonsdorf et al., 2017). However, note that the terms "*conditioned/unconditioned stimulus*" are labels that result from a slight mistranslation from the original Russian names (Todes, 2014; Vervliet & Boddez, 2020). The terms *conditional* and *unconditional* would actually be more appropriate. The CS elicits a given conditioned response only *conditionally* after it has been paired with the US, which generates the unconditioned response *unconditionally*. Nevertheless, the terms *conditioned* stimulus and *unconditioned* stimulus are used throughout the present thesis. These labels have become scientific terms over the years and this wording is used most frequently in the current literature. However, it should be kept in mind that conditioning refers to stimuli that become *connected* through learning.

One hundred years ago, in 1920, John B. Watson and Rosalie Rayner published the *first* human fear conditioning study in the *Journal of Experimental Psychology*. This work became famous as the "Little Albert" study (Watson & Rayner, 1920). Its 100th anniversary has recently been celebrated with a Special Issue on "Memories of 100 Years of Human Fear Conditioning Research and Expectations for its Future," which appeared in the journal *Behaviour Research and Therapy* (Vervliet & Boddez, 2020). The continued interest in this work underlines the groundbreaking and innovative nature of this seminal study. Despite ethical concerns raised later (for a current discussion, see Fridlund et al., 2020), this publication has had a tremendous impact on experimental and clinical psychology. The 11-month-old child "Albert," who was an inpatient at Johns Hopkins University Hospital in Baltimore, did not show any fear when he was confronted with a white rat. After this rat (i.e., the CS) had been paired with an aversive noise (i.e., the US) a few times, Albert acquired a robust fear of this rat, which was interpreted as successful conditioning. Although this demonstration of successful fear acquisition to an initially innocuous stimulus (i.e., the rat) has been considered the first fear conditioning experiment in humans, it should be kept in mind that, using today's terminology, this procedure is not an example of "pure"

classical conditioning. Note that the unpleasant noise was administered when Albert's hand touched the animal. Thus, the experiment also included operant learning procedures. In the subsequent years, the observations of Watson and Rayner (1920) have been elaborated with more sophisticated experimental designs (Lonsdorf et al., 2017; Milad & Quirk, 2012). Underlying behavioral and neural processes, for example, have been explored in humans and animals (Haaker et al., 2019). Until now, an exponentially growing number of studies have used the fear conditioning paradigm to investigate learning mechanisms that are relevant for etiological models of pathological fear (Fanselow & Sterlace, 2014). Watson and Rayner (1920) already mentioned interest in procedures to treat or "unlearn" fear, but they did not have the opportunity, as Albert was dismissed from the hospital. Later, extinction learning (i.e., showing the CS repeatedly without US presentation) has been identified as an extremely effective way to reduce fear and defensive behavior (Craske et al., 2017; Milad & Quirk, 2012). This line of research has led to the development of novel and excellent methods in the treatment of clinical fear (Fullana et al., 2020; Lipp et al., 2020).

The "Little Albert" study inspired a large body of research, and even 100 years later, fear conditioning continues to be a core paradigm for psychological research (Vervliet & Boddez, 2020). However, a closer look at this early and seminal "Little Albert" study reveals major methodological pitfalls. For example, stimulus presentation was controlled by Watson and Rayner themselves, and it cannot be entirely excluded that not only the white rat, but also one of the experimenters became a fear-provoking CS. More recent research has overcome several initial problems and has developed sophisticated fear conditioning paradigms that allow investigating fear-related processes in humans in a much more standardized way (Lonsdorf et al., 2017).

Modern paradigms for human fear conditioning typically include several *experimental stages* (Lonsdorf et al., 2017). In each stage, stimulus presentation is controlled in a highly elaborated way, an approach that ensures high internal validity regarding the interpretation of underlying learning mechanisms (see Figure 3). All fear conditioning studies that are part of the present thesis contain such experimental stages, which are therefore described in more detail.

During an initial *habituation phase* (sometimes called "pre-acquisition"), innocuous sensory stimuli (the stimuli that later become *conditioned* stimuli) are shown a few times to familiarize participants with the stimuli. Furthermore, the implementation of a habituation phase allows researchers to test explicitly whether responses to the CSs (see next paragraph) were similar before conditioning. The majority of human fear conditioning studies (including all studies of the present thesis) employ visual cues as CS, for example, pictures of neutral human faces.

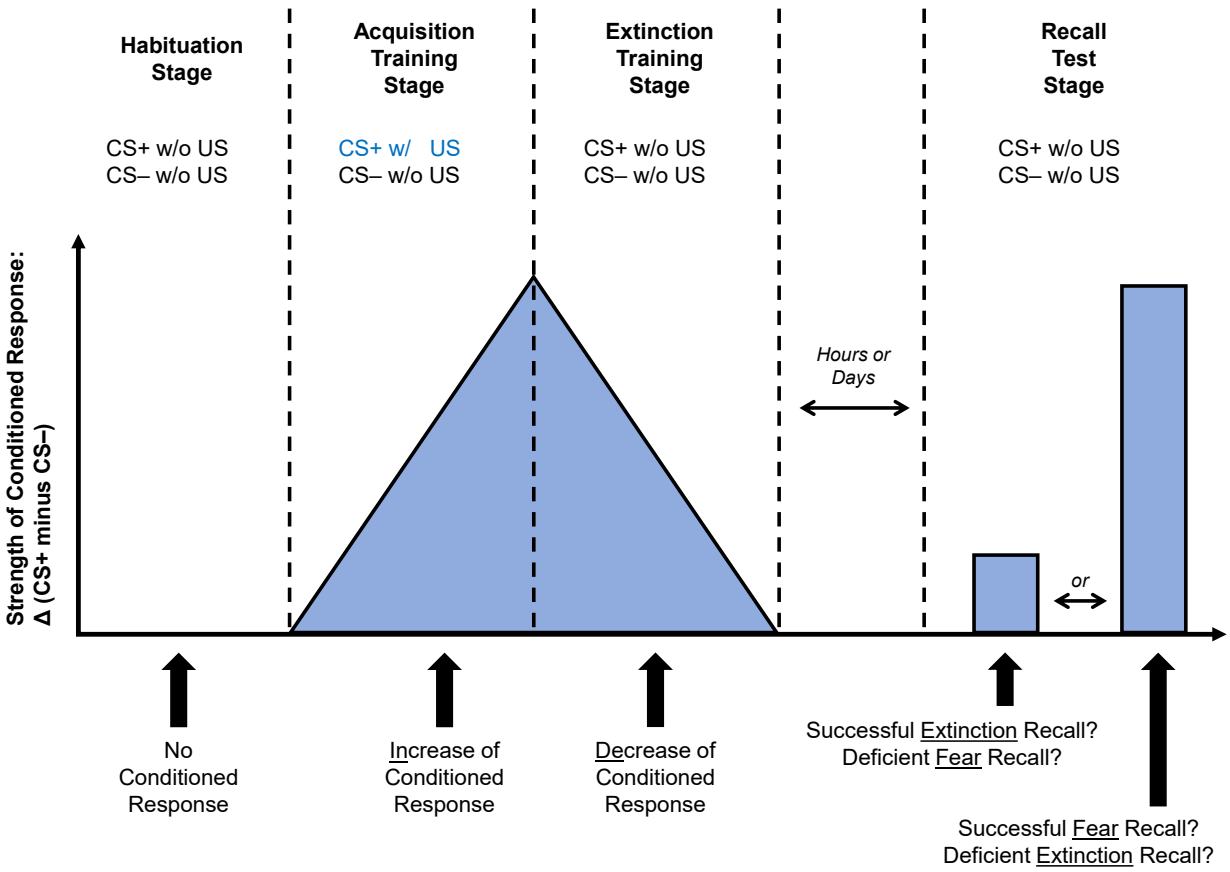


Figure 3. Experimental stages of typical fear conditioning paradigms in humans. During a *habituation* stage, all conditioned stimuli (CSs) are shown without ("w/o") the unconditioned stimulus (US) to familiarize participants with the stimuli. During the *acquisition training* stage, the CS+ is paired with ("w/") an unpleasant US, while the CS- is never paired. Afterward, during the *extinction training* stage, the CS+ and CS- are both shown without US presentation. Differential conditioned responses (CS+ minus CS-) should increase and decrease during acquisition and extinction, respectively. The robustness of fear and extinction recall can be tested during a *recall test* stage, which takes place several hours or days after fear acquisition and extinction.

The habituation phase is usually followed by an *acquisition training phase*. During this stage, the CS (at least one CS type, see below) is repeatedly paired with an unpleasant US (e.g., a percutaneous electrical stimulation or an aversive noise). Thereby, participants acquire "fear" of the CS. The "Little Albert" experiment described above can be conceptualized as a "single-cue" protocol, as fear was conditioned to *one* CS (the white rat). To gain statistical power and to control for non-associative processes in responding to the stimuli (e.g., orienting, habituation), modern conditioning studies usually apply a differential protocol (Lonsdorf et al., 2017). In differential fear conditioning paradigms, one CS (the CS+) is paired with the aversive US, while a second CS (the CS-) remains unpaired. The "differential fear response" (i.e., the conditioned response) can be quantified as a larger threat response to CS+ compared with CS-. The CS+ usually co-terminates

with the noxious US, and the onset of the US is often delayed to the CS onset ("delay-conditioning"; Lonsdorf et al., 2017). During acquisition training, the CS+ (but not the CS-, which acts as a safety signal) continuously acquires aversive properties.

In most fear conditioning studies, researchers are not only interested in fear acquisition, but also in extinction processes (Milad & Quirk, 2012). During an *extinction training phase*, unreinforced CS+ and CS- are presented. This means that both CSs are shown without any US presentation. Due to the absence of the aversive US, a new extinction memory trace gets established, and the differential fear response (CS+ minus CS-) diminishes gradually (Furini et al., 2014). Figure 3 illustrates how conditioned responses are assumed to increase and to decrease during fear acquisition and extinction, respectively.

In many conditioning studies, the temporal stability of conditioned and extinguished fear responses is of particular interest (Quirk & Mueller, 2008). This is especially the case for clinical applications, as pathological fear is usually resistant to extinction and shows relapse frequently (Duits et al., 2015). Without appropriate treatment, exaggerated fear in anxiety disorders often does not fade enough over time. It can persist and interfere with normal functioning for years (Craske & Stein, 2016; Lenze & Wetherell, 2011). To assess the stability of conditioned and extinguished fear, several studies add a *recall test stage* (sometimes called "retention test"), during which the CSs are again presented in the absence of the US. This recall test stage can take place several hours or days after fear acquisition and extinction (Vervliet et al., 2013). Note that extinction training and recall test stages are often entirely identical. Thus, within-session extinction processes are likely to occur also during recall test stages, although this is often not intended and taken into account. As a further problem, if standard paradigms are used, it is almost impossible to separate *fear recall* from *extinction recall* (Lonsdorf et al., 2017). Specifically, a small conditioned response during a testing session could be either interpreted as successful extinction recall or deficient fear recall (see Figure 3). Conversely, a large conditioned response could be linked to the dominance of fear recall or the absence of extinction recall. In four studies of the present thesis (manuscripts 1, 4, 5, and 6), we aimed to separate fear recall from extinction recall. Therefore, we applied a specific experimental paradigm (Mueller et al., 2014) that allows researchers to disentangle both processes properly. This design is described in more detail in chapter 2.1.

Taken together, fear conditioning explains how animals and humans learn about threat. It is a basic learning procedure that describes how associations are built between danger and related cues in the environment (Vervliet et al., 2012). Notably, fear conditioning is an example of translational success. This paradigm has been applied in various animal models, healthy humans, and also

patients (Ressler, 2020). While fear conditioning is a core learning process that contributes to the etiology of anxiety and stressor-related disorders (Pittig et al., 2018), fear extinction is critical for the treatment of clinical fear (Craske et al., 2018). Animal research has culminated in the development of neurobiological models (Levy & Schiller, 2021) for threat processing (see next pages, chapter 1.2), which have been further translated to human research (Haaker et al., 2019) and used to improve the efficacy of exposure therapy (Fullana et al., 2020; Ressler, 2020).

1.2 From "Little Albert" to Neuroscience: Animal Research Gives Rise to Brain Models of Threat

To clarify terminology regarding threat processing, the "two-system" framework (as introduced by LeDoux and colleagues: LeDoux, 2017; LeDoux & Daw, 2018; LeDoux & Pine, 2016) has already been described at the beginning of this thesis. Although this conceptualization contains broad assumptions about underlying neural circuits, it remains relatively vague about specific brain areas.

Research in animal models has furthered our understanding of neural threat processing and has led to the development of neurobiological models of threat and fear processing (Adolphs, 2013; Calhoon & Tye, 2015; Hartley & Phelps, 2010; Levy & Schiller, 2021; Mattera et al., 2020; McCullough et al., 2016; Pitman et al., 2012; Ressler & Maren, 2019; Tovote et al., 2015; VanElzakker et al., 2014). Neural circuits involved in fear acquisition and extinction seem to be well conserved across evolution (Janak & Tye, 2015). Findings obtained from animal research can therefore inform knowledge about threat processing in humans. In the past years, the "traditional model" of threat processing (Amorapanth et al., 2000) has been published several times with slight modifications, including very recent summaries by Ressler and Maren (2019), as well as by Levy and Schiller (2021).

According to this model, Pavlovian fear conditioning is associated with widespread synaptic plasticity in the brain. When auditory stimuli are used as CSs (which is often the case in rodent studies), thalamic pathways project to the auditory cortex (Medina et al., 2002). In humans, visual stimuli are typically used as CSs, activating visual cortical areas (Miskovic & Keil, 2012; Stegmann et al., 2020; Thigpen et al., 2017). As illustrated in Figure 4, the amygdala plays a very prominent role in "traditional" models for threat processing. The amygdala receives sensory input through indirect thalamo-cortico-amygdala and direct thalamo-amygdala pathways (LeDoux, 2000). Sensory information about the CS-US contingency enters the amygdala through its lateral nucleus (LA), and the LA further projects to the central nucleus (CEA), which regulates peripheral conditioned responses and defensive behavior through output to the hypothalamus and brainstem regions (Hartley et al., 2014; Levy & Schiller, 2021). After CS-US pairings, output responses are also evoked by the CS alone due to conditioning-induced plasticity and long-term potentiation of synaptic transmission in the LA, but also in other amygdaloid nuclei (Bauer et al., 2001; Tovote et al., 2015).

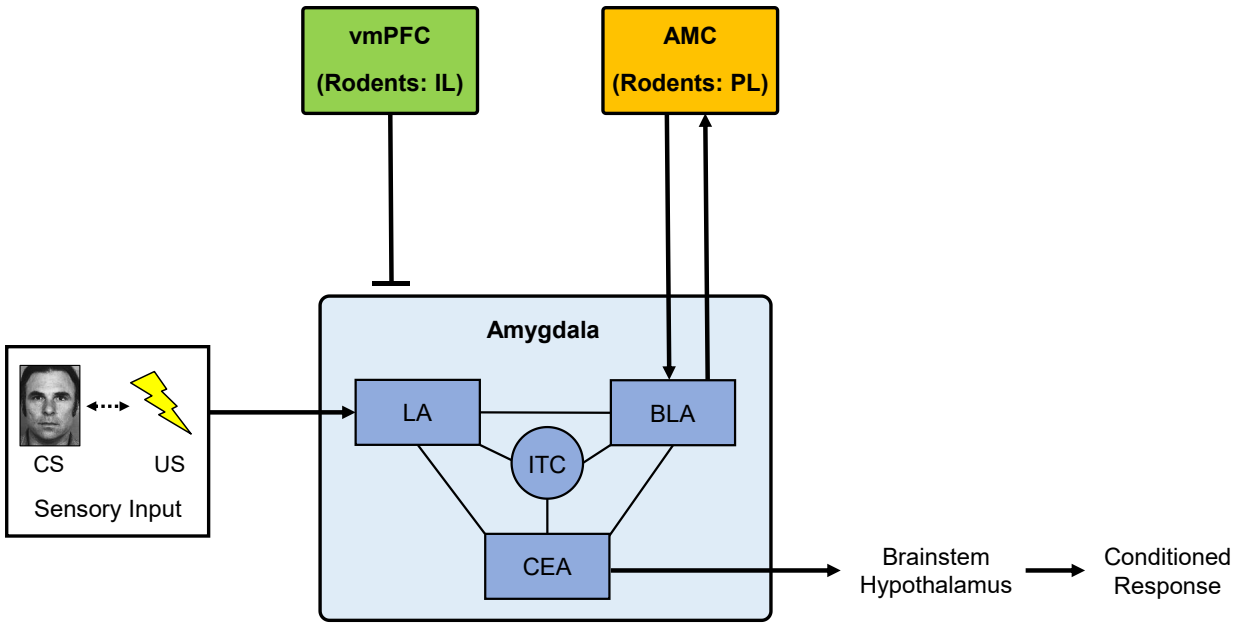


Figure 4. The "traditional" neurobiological model of fear conditioning and extinction (as proposed by several publications, e.g., Adolphs, 2013; Calhoon & Tye, 2015; Hartley & Phelps, 2010; Levy & Schiller, 2021; McCullough et al., 2016; Pitman et al., 2012; Ressler & Maren, 2019; Tovote et al., 2015). Sensory input about the contingency between the conditioned (CS) and unconditioned (US) stimuli enters the amygdala at the lateral nucleus (LA), and is further processed through the basolateral (BLA) and central (CEA) nuclei. The amygdaloid nuclei are connected with intercalated cell masses (ITC). Fear expression and extinction are regulated by the anterior midcingulate cortex (AMC; homolog area in rodents: prelimbic cortex, PL) and the ventromedial prefrontal cortex (vmPFC; homolog area in rodents: infralimbic cortex, IL), respectively.

The duration of amygdala responses evoked by the CS is very short (Goosens & Maren, 2004; Quirk et al., 1995) and lasts for only a few hundred milliseconds (Levy & Schiller, 2021). Hence, amygdalar activity cannot fully explain sustained responses to threat (e.g., freezing in rodents), which typically last for several seconds (Burgos-Robles et al., 2009). The prelimbic cortex (PL), which is considered the rodent homolog (Milad & Quirk, 2012) of the human anterior midcingulate cortex (AMC), seems to play a crucial role in maintaining *sustained* threat responses (Levy & Schiller, 2021; Pitman et al., 2012). Theta synchrony (Gilmartin et al., 2014) may be highly relevant for bidirectional connectivity between the PL/AMC and the basolateral nucleus (BLA) of the amygdala. In manuscript 6 of the present thesis (Sperl et al., 2019), the critical role of oscillatory theta activity for communication between these brain areas is translated into human research using simultaneously acquired electroencephalography (EEG) and functional magnetic resonance imaging (fMRI). Note that the AMC is (imprecisely) labeled as the dorsal anterior

cingulate cortex (dACC) in some publications (Milad & Quirk, 2012). Anatomically, the dACC can be divided into a relatively anterior and a rather posterior portion (Etkin et al., 2011). The localization of the *anterior* dACC is similar to the AMC (Vogt et al., 2003). In the literature, the terminology regarding this brain area is often vague. Specifically, the entire dACC is often equated with the AMC (e.g., Asemi et al., 2015), and, in most publications, the dACC is not further divided into anterior or posterior regions (Milad & Quirk, 2012). However, Vogt et al. (2003) demonstrated structural and functional differences between anterior and posterior parts of the midcingulate cortex. In the present thesis, the term *AMC* is used consistently, as this is a more precise description of the sub-region that is of particular relevance for threat processing (Etkin et al., 2011; Vogt et al., 2003). Nevertheless, when our results are compared with other studies, it should be kept in mind that the label "dACC" is often used in the literature when referring to this region.

Fear extinction can be conceptualized as "learned inhibition" of threat memories, which have been previously acquired through conditioning procedures (Furini et al., 2014). Neurobiologically, the rodent infralimbic cortex (IL), which is the putative homolog (Milad & Quirk, 2012) of the human ventromedial prefrontal cortex (vmPFC), is thought to inhibit fear expression through connectivity with the amygdala (Levy & Schiller, 2021; Luchkina & Bolshakov, 2019; Pitman et al., 2012). The specific actions of projections from the IL/vmPFC to amygdaloid nuclei are not further specified in Figure 4, as different theories have been proposed, and several parallel mechanisms may be involved (Bauer & Paré, 2016). A prominent theory (Pare et al., 2004) suggests that the IL/vmPFC might inhibit the CEA through projections to so-called intercalated cell masses (ITC). These inhibitory neurons are situated between the BLA and the CEA and control amygdala activity as a "switch off" system (Levy & Schiller, 2021). According to another theory, the IL/vmPFC seems to activate inhibitory interneurons in the LA (Rosenkranz et al., 2003). Connectivity between the IL/vmPFC and the BLA has also been discussed (Levy & Schiller, 2021). Whatever the exact mechanism may be, the IL/vmPFC seems to play an important role in extinction learning and the inhibition of conditioned threat responses.

The "traditional" model of neurobiological threat processing has often been criticized as an oversimplification (e.g., Alexander et al., 2020). Instead, brain areas implicated in threat processing show massive connectivity (McMenamin et al., 2014; Pessoa et al., 2019; Sylvester et al., 2020). Contextual information, for example, is assumed to be processed through projections from the hippocampus (Chaaya et al., 2018). Contextual modulations are not in the central focus of the present thesis, and, for simplicity, the hippocampus is therefore not drawn in Figure 4. Furthermore, sensory brain regions also play important roles in threat learning (Miskovic & Keil, 2012). Notably, fear conditioning sharpens the tuning of visuocortical neurons (Stegmann et al.,

2020) and facilitates plasticity in already early visual neurons (Thigpen et al., 2017). These findings are not sufficiently taken into account in the "traditional" threat model (see Figure 4), where neural conditioning processes seem to start later, namely in the LA. In manuscript 3 of the present thesis (Sperl et al., 2021), we address this weakness of the "traditional" model and explore how fear conditioning affects very early (already 33–60 ms after CS onset) neural processing. We further elucidate how these short-latency neural responses evolve throughout fear acquisition.

As a further limitation, brainstem regions are often perceived as rather undifferentiated segments in the "traditional" model. These regions are typically exclusively linked to the organization of peripheral physiological and behavioral outputs. It must be emphasized that this basic view of the brainstem is outdated. It has been shown that brainstem structures (e.g., the periaqueductal gray; PAG) show *reciprocal* connectivity with several regions of the "traditional" threat network (George et al., 2019; Watson et al., 2016). Thus, the conceptualization of the brainstem as a "basic output structure" is wrong. In manuscript 4 of the present thesis (Sperl et al., submitted to *Neuropsychopharmacology*), we further close this gap between brainstem and cortical structures. Specifically, we explore how noradrenaline (norepinephrine; NE) release from the locus coeruleus (Poe et al., 2020) – a phylogenetically conserved small brainstem nucleus – dynamically modulates EEG correlates of fear consolidation and extinction learning.

The "two-system" framework of LeDoux and colleagues (LeDoux, 2017; LeDoux & Daw, 2018; LeDoux & Pine, 2016) has been described at the beginning of this thesis. According to this model, the conscious feeling of "fear" is linked to activity in cortical brain areas, while peripheral physiological responses and defensive behavior are mostly generated by subcortical circuits. This duality is not taken into account by the "traditional" neurobiological model (including current publications of this model, e.g., Levy & Schiller, 2021), where output is generated solely through projections from the CEA to the brainstem and hypothalamus (see Figure 4). If the mental state of "fear" is not differentiated adequately from physiological and behavioral responses, translation of results from animal research to human applications can be problematic and lead to contradictory conclusions. This problem has been explained in more detail by LeDoux and Pine (2016). For example, the efficacy of so-called "anxiolytic" medications (which is a rather misleading label itself, according to the "two-system" framework) is often measured with physiological and behavioral responses in rodents (which are supposed to be mediated by subcortical circuits). In sharp contrast, clinicians usually evaluate reductions in pathological fear based solely on self-report measures (which are supposed to be mediated by cortical circuits). If a novel medication successfully reduces threat-evoked changes in physiology and behavior but does not affect the mental state of fear, improvements are only of limited clinical relevance. Scientific progress in this

regard often remains disappointing if the duality of cortical and subcortical networks is not considered adequately (LeDoux & Pine, 2016). In the manuscripts of the present thesis, the term "conditioned *fear* response" is often used in a broad way, including also physiological measures (e.g., changes in skin conductance or heart rate). Note that the differentiation of "fear" versus "defensive physiological adjustments and behavior" (as elaborated above, see the very first part of chapter 1 and the illustration in Figure 1) has been derived from a very recent, evolving discussion. In other words, the revised terminology has been developed in parallel to the preparation of the studies of this thesis. It should be noted that a precise differentiation between mental and physiological/behavioral processes is of high relevance for the translation of animal research. However, this issue is considerably less problematic for human research. In the studies of the current thesis, conditioned responses were not only measured with physiological methods (e.g., skin conductance, heart rate, EEG, and fMRI), but also with affective ratings of the CSs. These affective ratings typically assess the CS-associated arousal (e.g., "How aroused are you when looking at the CS+/CS-?"), valence (e.g., "How good or bad do you feel when looking at the CS+/CS-?"), and fear (e.g., "How much fear do you have when looking at the CS+/CS-?"). It is obvious that such affective ratings cannot be applied in animal research. If, however, the CS+ (versus CS-) does not only evoke physiological adjustments, but also changes in arousal, valence, or even explicit fear ratings, it is better justified to interpret elevated physiological responses (e.g., heightened skin conductance responses [SCRs], changes in EEG signatures) as conditioned *fear* measures.

The model in Figure 4 implies that threatening stimuli are processed in a similar way in "every" brain, and individual differences are not further specified. However, it is well known that people differ in their neural, peripheral physiological, behavioral, and subjective responses to threats (Lonsdorf & Merz, 2017). In manuscript 5 of the present thesis (Panitz et al., 2018), we investigate the influence of catecholaminergic gene polymorphisms and personality traits (see also chapter 3.3 in the discussion section) on fear conditioning and extinction.

The "traditional" model proposes that the US consists of a physical stimulus (e.g., an unpleasant electric shock), which (through sensory input, see Figure 4) activates modality-specific sensory brain areas (e.g., the somatosensory cortex for electrical stimulation; Medina et al., 2002) and is further processed in the LA (Levy & Schiller, 2021). However, it can be debated whether some stimuli are better suited to serve as a US than others. In manuscript 1 of the present thesis (Sperl et al., 2016), we probe whether an electric shock US or a white noise US evokes larger conditioned responses in a fear conditioning paradigm that is optimized for EEG research and contains a relatively high number of trials. Furthermore, it remains unclear whether the US really needs to be

a *physical* stimulation. The question that arises is whether an aversive *imagination* would also be sufficient. This issue is addressed in manuscript 2 of the present thesis (Mueller, Sperl, & Panitz, 2019).

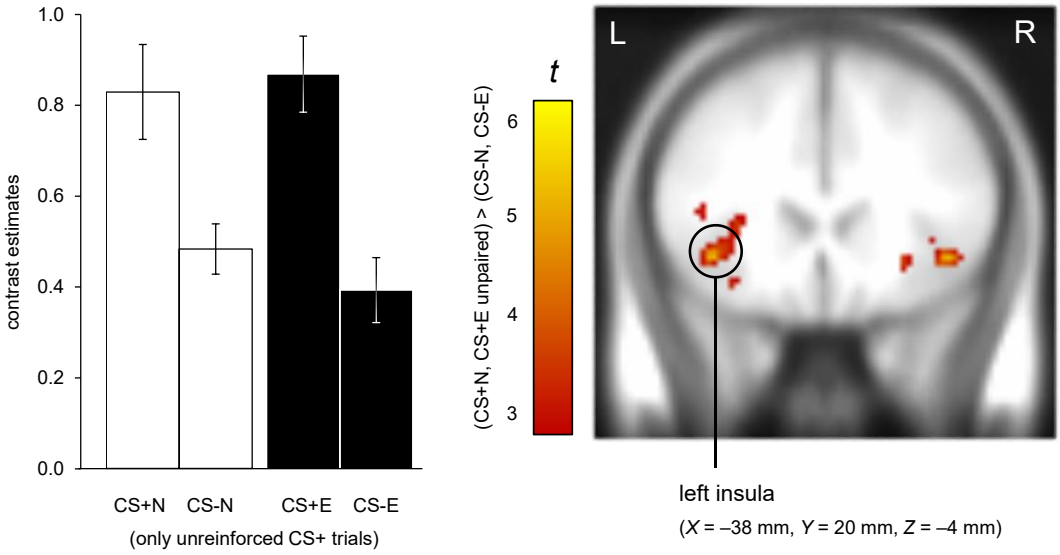
1.3 Fear Conditioning in Humans: fMRI as a Tool to Translate Neurobiological Knowledge?

Functional magnetic resonance imaging (fMRI) has been widely used to translate knowledge about brain correlates of fear conditioning from animal research into the human realm. In line with the "two-system" framework suggested by LeDoux and colleagues (LeDoux, 2017; LeDoux & Daw, 2018; LeDoux & Pine, 2016), fMRI research in humans has revealed that conditioned threat cues recruit an extensive brain network of cortical and subcortical structures (Biggs et al., 2020; Fullana et al., 2016). Fear conditioning and fear expression have been linked to increased activation in the amygdala (Chauret et al., 2019; Kim & Jung, 2006; Sehlmeyer et al., 2009; but: Fullana et al., 2016), insula (Biggs et al., 2020; Fullana et al., 2016; Sehlmeyer et al., 2009), and AMC (Fullana et al., 2016; Milad, Quirk et al., 2007; Sehlmeyer et al., 2009). Although the amygdala plays the most prominent role in "traditional" models (see Figure 4), which are often derived from animal research (Ressler & Maren, 2019), the most recent and sophisticated meta-analysis on fMRI fear conditioning studies in humans (Fullana et al., 2016) was – surprisingly – unable to confirm robust amygdala activations during fear acquisition. Conversely, consistent activations of the insula seem to be among the most reliable findings from human fear conditioning studies (Fullana et al., 2016), but this structure does not play a key role in "traditional" neurobiological models of threat processing, as illustrated in Figure 4. In manuscript 6 of this thesis (Sperl et al., 2019), we confirmed significantly enhanced insula activation for CS+ compared with CS- during fear acquisition (see Figure 5A). Altogether, there seems to be a gap between animal-based models and evidence from human fMRI studies. On the one hand, animal models suggest that the amygdala is the *key* structure (Levy & Schiller, 2021; Ressler & Maren, 2019), but fMRI research cannot replicate this finding in humans reliably. On the other hand, fear acquisition consistently activates the insula in human fMRI studies (Fullana et al., 2016). Still, the insula seems to play a rather peripheral and subordinate role in "traditional" models. This divergence suggests that (a) "traditional" models may need to be revised and/or (b) methodology in humans needs to be improved. A critical methodological challenge, which might explain the lack of robust amygdala effects in the meta-analysis of Fullana et al. (2016), is the observation that amygdala activity shows a rapid habituation over time (Armony & Dolan, 2001; Büchel et al., 1998; Büchel et al., 1999; Yin et al., 2018). If this response pattern is not addressed (e.g., when amygdala responses are averaged over a large number of trials), "real" effects may be missed. Further, if many studies that do not adequately control for amygdala habituation are included in a meta-analysis, "real" amygdala activation (which should be particularly/only pronounced during *early* acquisition trials) may be overlooked, and wrong conclusions could be drawn. Specifically, Yin et

al. (2018) emphasized that the lack of amygdala effects in several fear conditioning studies (Fullana et al., 2016) may presumably be related to the adaptation of amygdaloid responses, which occurs over the course of conditioning trials. Previous studies suggest that amygdala habituation can be best described by an exponentially decaying function (Armony & Dolan, 2001; Büchel et al., 1998; Büchel et al., 1999). In manuscript 6 of this thesis (Sperl et al., 2019), we were specifically interested in amygdala activation during a fear and extinction recall test that took place 24 hours after acquisition and extinction stages. To account for amygdala habituation, recall test trials were weighted with an exponentially decaying function. This function was derived from the habituation of SCRs, given that there is evidence that habituation of SCRs (Lonsdorf et al., 2017; Sperl et al., 2016) is correlated with habituation of amygdala activation (Büchel et al., 1998; Knight et al., 2005; Phelps et al., 2004). Importantly, this analysis – which explicitly accounted for the adaptation of amygdala responses – demonstrated elevated amygdala activation (see Figure 5B) for stimuli that have been fear-conditioned and not extinguished (Sperl et al., 2019). As expected, conditioned amygdala responses diminished across trials. Note that no amygdala effects could be detected when all trials were weighted equally (i.e., when the analysis did not account for habituation processes), which could explain the absence of robust amygdala effects in the meta-analysis by Fullana et al. (2016).

Regarding extinction learning and extinction recall, human fMRI studies have revealed elevated activation in the vmPFC (Hermann et al., 2016; Kalisch et al., 2006; Milad & Quirk, 2012; Milad, Wright et al., 2007) and decreased activation in the amygdala (Hermann et al., 2016; Phelps et al., 2004; Sehlmeyer et al., 2011), findings that are consistent with the neurobiological model derived from animal research (see Figure 4). However, there are also inconsistent and diverging results. Fear extinction has not only been associated with reduced (Hermann et al., 2016; Phelps et al., 2004; Sehlmeyer et al., 2011), but also with elevated amygdala activation (Gottfried & Dolan, 2004; LaBar et al., 1998; Milad, Wright et al., 2007). Similarly to the inconsistency of amygdalar effects during fear acquisition and extinction, the most recent meta-analysis of fMRI studies during human fear extinction (Fullana et al., 2018) could not confirm a robust association between fear extinction and vmPFC activation (although there is "nuanced" support for vmPFC contributions, which seem to depend on paradigm characteristics; this issue has been further discussed by Morriss et al., 2018). In manuscript 6 of the present thesis (Sperl et al., 2019), we provide initial evidence that the contribution of the vmPFC may be related to individual differences in threat processing. Remarkably, we demonstrated that extinction recall was associated with vmPFC activation (see Supplementary Material F of manuscript 6, chapter 5.6), but this activation pattern was negatively

A Left Insula BOLD Responses during Fear Conditioning (Day 1)



B Left Amygdala BOLD Responses during Fear and Extinction Recall (Day 2)

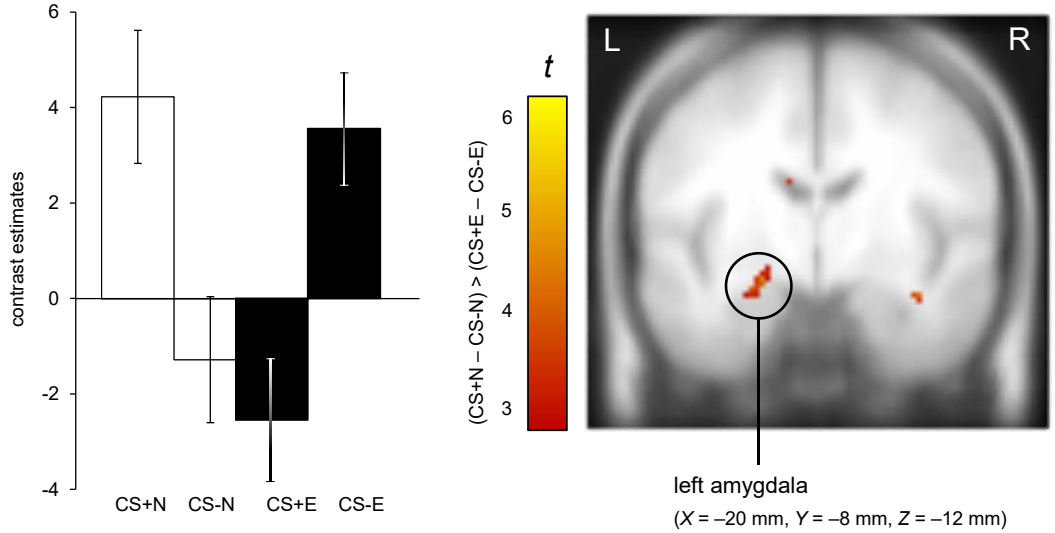


Figure 5. In manuscript 6 (Sperl et al., 2019), we recorded fMRI during fear conditioning and demonstrated insula and amygdala activations. During fear acquisition, CS+ (CS+N, CS+E) compared with CS- (CS-N, CS-E) evoked significantly enhanced activation in the left insula (A). Twenty-four hours later, we observed significantly enhanced activation in the left amygdala (B) for CS+, specifically for the nonextinguished CS+N (contrast: [CS+N – CS-N] > [CS+E – CS-E]). Amygdala activation could only be detected when habituation of amygdala activity was modeled by an exponentially decaying function, which was based on habituation of skin conductance responses (SCRs). CS+N and CS-N: nonextinguished stimuli; CS+E and CS-E: extinguished stimuli (for further design characteristics, see chapters 2.1 and 2.6). Note: For illustrative purposes, the intensity threshold was set to $p \leq .005$ (uncorrected) with a minimal cluster threshold of $k \geq 5$ contiguous significant voxels. Activations (t-values) were superimposed on the MNI305 T1 template. All coordinates (X, Y, Z) are given in Montreal Neurological Institute (MNI) space. "L" = left, "R" = right brain hemisphere. Bar graphs show the mean contrast estimates (± within-subject *SEM*, O'Brien & Cousineau, 2014) for a cluster of voxels with $p \leq .005$ (uncorrected) surrounding the peak voxel within the insula/amygdala region of interest (ROI). Figure republished from Sperl et al. (2019).

correlated with oscillatory theta activity (measured with EEG at a frontomedial channel). In other words: This between-subjects correlation indicates that subjects with relatively suppressed AMC-related frontomedial theta power to extinguished (versus nonextinguished) threat stimuli are characterized by relatively strong vmPFC activation to those stimuli. This result pattern would be consistent with the "traditional" neurobiological threat model (see Figure 4), which suggests opposite effects of vmPFC and AMC regions on "fear" expression. A similar (but – as expected – in the opposite direction, which is also consistent with the "traditional" neurobiological model) correlation was observed for amygdala activity and theta oscillations. This dynamic interplay is further addressed in chapters 1.4 and 2.6. The sample size of our study is too small to assess individual differences or personality measures in more detail, but we provide initial support for the hypothesis that differences between participants could be critical for the robustness and interpretation of fMRI results (Lonsdorf & Merz, 2017).

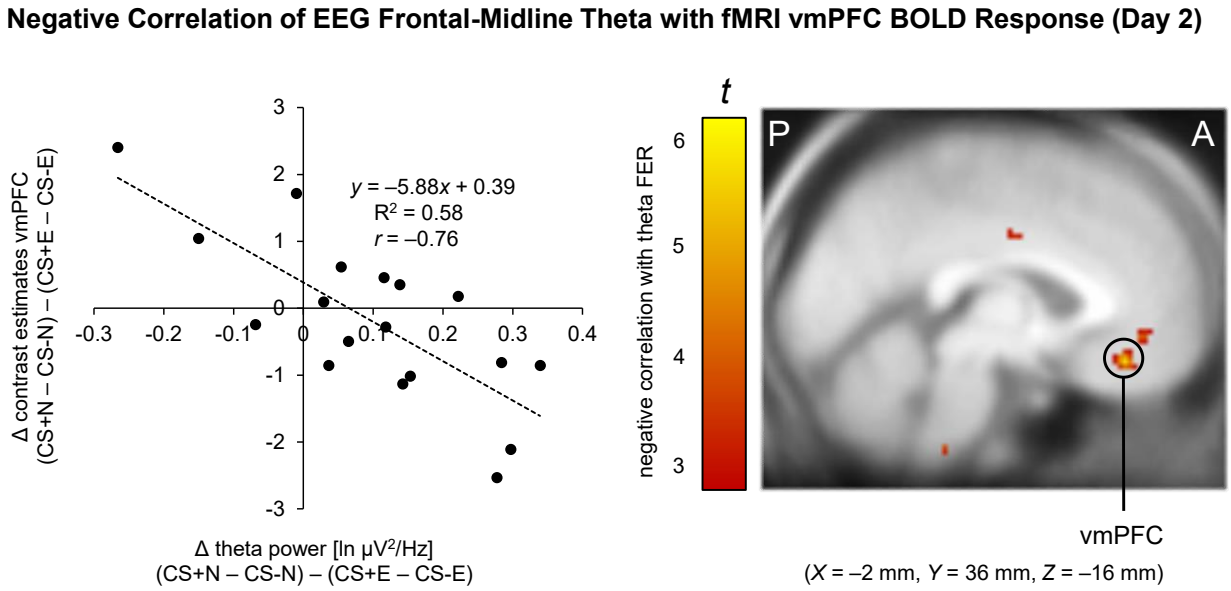


Figure 6. In manuscript 6 (Sperl et al., 2019), we recorded simultaneous EEG-fMRI and demonstrated a negative correlation (between-subjects correlation, $p = .038$) between frontomedial theta activity (measured at channel Fz) and ventromedial prefrontal cortex (vmPFC) activation for the contrast [CS+N – CS-N] > [CS+E – CS-E] during a recall test, which took place 24 hours after acquisition and extinction. CS+N and CS-N: nonextinguished stimuli; CS+E and CS-E: extinguished stimuli (for further design characteristics, see chapters 2.1 and 2.6). Note: For illustrative purposes, the intensity threshold was set to $p \leq .005$ (uncorrected) with a minimal cluster threshold of $k \geq 5$ contiguous significant voxels. Activations (t-values) were superimposed on the MNI305 T1 template. All coordinates (X, Y, Z) are given in Montreal Neurological Institute (MNI) space. A = anterior, P = posterior. Figure republished from Sperl et al. (2019).

Taken together, the evidence from human fMRI fear conditioning studies has been mixed and, to some extent, contradictory. Some studies (especially older publications) have pointed to a

prominent role of the amygdala and vmPFC for fear acquisition and fear extinction, respectively. However, more recent meta-analyses (Fullana et al., 2016; Fullana et al., 2018) show a rather inconsistent overall picture and cannot confirm a robust contribution of these brain regions. Various reasons may be responsible for these discrepant findings. One possible explanation could be derived from the so-called "two-system" framework (LeDoux, 2017; LeDoux & Daw, 2018; LeDoux & Pine, 2016), which has been explained at the beginning of this thesis. We may speculate that subcortical circuits (including the amygdala, which generates defensive peripheral physiological adjustments and behavioral responses to threat) could be more important in animals. Specifically, subcortical circuits seem to be particularly conserved across species (Janak & Tye, 2015). By contrast, cortical circuits (which generate a conscious feeling of "fear") might be more relevant in humans. This divergence might explain the lack of robust amygdala activations in human fMRI studies (Fullana et al., 2016). In fact, experimental setups in humans (which typically include interaction with a "friendly" investigator) seem to be less "life-threatening" than typical conditioning paradigms in animals; therefore, amygdala-related processes and defensive neural circuits may be recruited to a lesser extent. This line of reasoning would suggest that involved neural circuits actually differ in animals and humans. However, it is also conceivable that methods available in humans are not sufficiently suited to detect reliably amygdala and vmPFC activation during threat. For example, small and adjacent subnuclei of the amygdala have differential functions with regard to the expression versus inhibition of conditioned threat (Quirk & Mueller, 2008), and activation from these subnuclei is difficult to disentangle with fMRI in humans (Keifer et al., 2015). As explained above, the temporal characteristics of amygdalar activity have often been ignored, which may also be responsible for the lack of amygdala effects in several fMRI studies (Fullana et al., 2016). In manuscript 6, we demonstrated that amygdala activation can be detected during fear/extinction recall if its rapid habituation curve is taken into account (Sperl et al., 2019). It was not the primary goal of our study to explore temporal dynamics of amygdala effects. We aimed to explore the interplay of amygdala activity and prefrontal oscillations in the theta band (see chapter 1.4). For this purpose, however, it is a prerequisite to detect amygdala activation reliably. Therefore, we had to model explicitly the habituation curve of this brain region. Finally, our results on the interplay of theta oscillations and vmPFC activity suggest that individual differences may also be relevant and further explain the lack of vmPFC effects in studies on fear extinction (Fullana et al., 2018). For successful translation of animal findings into humans, adequate methods are necessary in both fields. Our approach and results (e.g., emphasizing the habituation of amygdala activity) aim to improve existing methods for human research.

1.4 fMRI is not Enough?! The Necessity of EEG for the Understanding of Neurophysiological *Processes* in Humans

Research methods that are available in animals (e.g., recording intracranial electrical activity of single units, optogenetics) have dramatically advanced our understanding of neural responses during threat processing. As described in chapter 1.2, this line of research has culminated in the development of neurobiological models, which allow deriving new hypotheses that can be further tested in animal and human studies. In particular, rodent research provides the possibility to assess neural responses to threats with high spatial and temporal precision (Fadok et al., 2017). To translate this neurobiological knowledge into the human field, fMRI has been the most prominent method, as outlined in chapter 1.3. Indeed, fMRI has been a beneficial tool to illuminate the anatomy of threat processing in the living human brain (Fullana et al., 2016, 2018). Specifically, fMRI offers excellent spatial resolution, allowing one to image also deep structures like the amygdala (Geissberger et al., 2020; Janak & Tye, 2015; Patin & Hurlemann, 2011), although distinct subnuclei are still difficult to differentiate (Keifer et al., 2015). Although fMRI is well suited to detect slower neural processes, the investigation of fast and rather transient brain processes requires other techniques that offer much higher temporal precision. EEG is a neuroscientific method that, in contrast to fMRI, provides the possibility to track neural activity with an extremely high resolution in the range of milliseconds (Hajcak et al., 2019; Miskovic & Keil, 2012). The incredible advantage of EEG to elucidate rapid brain responses toward threats has recently been illustrated by Mueller (2019), as shown in Figure 7. Remarkably, the latency of the human startle reflex (Blumenthal et al., 2005) and the duration of a complete predatory strike of a horned viper (Janoo & Gasc, 1992) are less than 400 ms, and the break reaction time in healthy car drivers (Tashiro et al., 2005) is less than 700 ms (see Figure 7A). To prevent car accidents, fast responses in the brain are required. However, as illustrated in Figure 7B, the temporal resolution of fMRI is too poor to capture sufficiently such fast threat-related processes (Hendriks et al., 2020). By contrast, EEG is a neuroscientific tool with an incredibly high temporal resolution that allows one to detect *rapid* neural processes to threat (Hajcak et al., 2019). As illustrated, such fast responses are crucial for survival and everyday life.

In the field of mental chronometry, EEG methods have been increasingly used (Posner, 2005). This approach aims to describe the time course of information processing through the nervous system (Gupta et al., 2019; MacNamara et al., 2013; Posner, 2005) and allows one to elucidate how threatening cues guide attention and processing speed (Bublatzky et al., 2010; Bublatzky & Schupp, 2012). After fear conditioning, prioritized processing of threat cues can occur at different

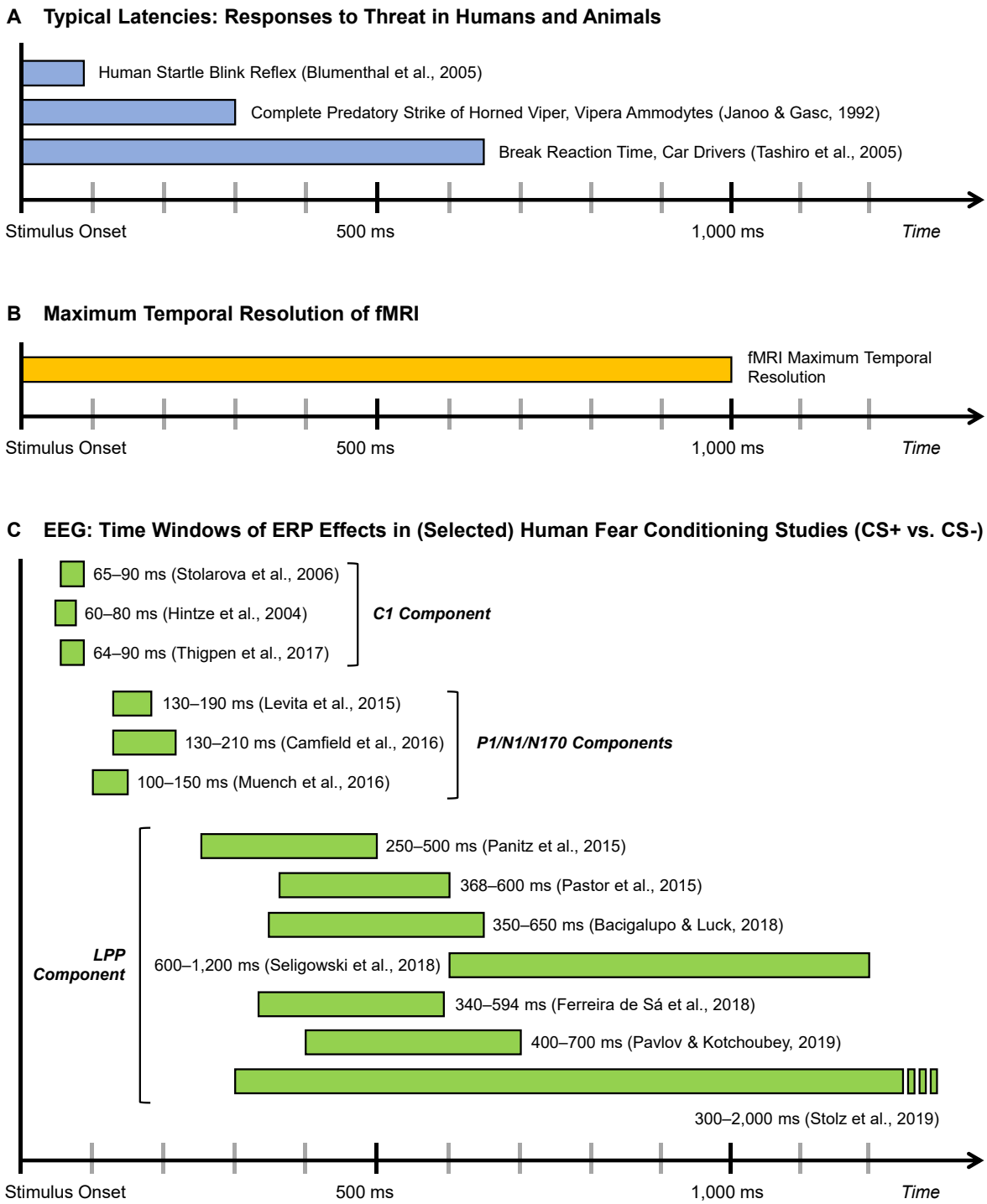


Figure 7. In a recent book chapter, Mueller (2019) illustrated the necessity of rapid neural processes toward threats. (A) The latency of the human startle reflex, the duration of a complete predatory strike of a horned viper, and the break reaction time in healthy, awake, young car drivers are relatively low (blue bars). (B) The speed of these threat-related responses is in remarkable contrast to the relatively poor temporal resolution of fMRI (orange bar; Hendriks et al., 2020). (C) By contrast, EEG provides the possibility to track neural activity in the range of milliseconds. Authors of previous fear conditioning studies have shown EEG modulations during the typical latencies of the C1 (~50–100 ms), P1/N1/N170 (~80–200 ms), and LPP (> ~300 ms) components (green bars; Miskovic & Keil, 2012; Wieser & Keil, 2020). The latencies during which fear conditioning effects have been observed are illustrated for some selected EEG studies. Note that Muench (2016) did not compare CS+ with CS-. Instead, a nonextinguished CS+ was compared with an extinguished CS+.

latencies after CS onset, affecting several sensory and cognitive stages (Gupta et al., 2019; Wieser & Keil, 2020). In particular, the visual event-related potential (ERP) comprises a series of characteristic voltage deflections and provides a powerful tool to unravel different sensory and motor stages of information processing after threat detection with millisecond-by-millisecond resolution (Hajcak et al., 2019; Hillyard & Anllo-Vento, 1998; Klumpp & Shankman, 2018). The ERP waveform can be obtained from EEG recordings through averaging across stimulus-locked epochs. This method offers critical insights into the human brain, which allows one to develop and to test models with regard to the temporal unfolding of cognitive and affective processes. Different EEG latencies may be linked to various processing stages in the "traditional" neurobiological model for threat processing (see Figure 4). Over several decades, there has been a debate in ERP and attention research regarding whether attentional selection occurs rather "early" or "late" after stimulus onset. Although this dichotomic division into pre-attentive and attentive processing has been shown to be problematic (Anderson, 2011; Eimer, 2018), it is possible that attentional modulation is responsible for specific functions at different stages of threat processing (Kastner & Pinsk, 2004). A crucial goal of attention is to facilitate and accelerate the detection of potential danger (Mogg & Bradley, 1998; Wieser & Keil, 2020). Sketching out relationships between specific ERP periods and various cognitive processes allows drawing inferences about how different processing stages are related to each other.

As illustrated above, EEG is an extraordinarily well-suited tool to unravel how threat guides several perceptual and evaluative processing stages in the human brain (Bublatzky & Schupp, 2012; Lang & Bradley, 2010; Miskovic & Keil, 2012; Wieser & Keil, 2020). ERP components with different latencies can be assessed to uncover relevant stages that are involved in neural threat processing. Figure 7C shows relevant time windows during which previous fear conditioning studies have reported differential modulations for conditioned stimuli. There is evidence (e.g., Hintze et al., 2014; Stolarova et al., 2006; Thigpen et al., 2017) that CS+ compared with CS- evokes larger parieto-occipital ERP amplitudes during the time window of the C1 component (~50–100 ms). Some magnetoencephalographic (MEG) studies suggest that fear-conditioned stimuli can modulate neural activity even earlier, during latencies that begin before 40 ms after CS onset (Bröckelmann et al., 2011; Kluge et al., 2011; Morel et al., 2012; Steinberg et al., 2013). These findings are consistent with the assumption of plasticity in very early cortical regions, which is based on findings from animal research (Miskovic & Keil, 2012; Thigpen et al., 2017). Specifically, it has been hypothesized that neurons in primary sensory areas alter their tuning behavior during fear conditioning, a phenomenon that selectively amplifies stimuli that have been paired with an aversive outcome (Bakin & Weinberger, 1990; Headley & Weinberger, 2015;

Weinberger, 2004). Some studies (e.g., Camfield et al., 2016; Levita et al., 2015; Muench et al., 2016) suggest that fear-conditioned stimuli amplify ERP responses during mid-latency components (roughly corresponding to the P1/N1/N170 components, as illustrated in Figure 7C), but other authors could not replicate these findings (e.g., Seligowski et al., 2018; Stolarova et al., 2006; Stolz et al., 2019). Under certain circumstances, attention could be elevated to both threat (CS+) and safety (CS-) cues, which may have led to these inconsistent findings during mid-latency time windows (Bublatzky & Schupp, 2012; Schindler & Bublatzky, 2020). There is a need to better understand under which conditions threat modulates which ERP components. For example, Muench et al. (2016) found more positive P1 amplitudes for fear-conditioned face stimuli, but only during a self-relevant threat context. The most consistent finding seems to be that fear conditioning modulates late-latency ERP responses. The late positive potential (LPP) is a slow ERP wave that starts around 300 ms after stimulus onset and is related to sustained attention due to stimulus significance (Cuthbert et al., 2000; Hajcak & Foti, 2020). Many studies have confirmed that CS+ compared with CS- is associated with reliably enhanced LPP amplitudes at parieto-occipital electrode sites (e.g., Bacigalupo & Luck, 2018; Ferreira de Sá et al., 2019; Panitz et al., 2015; Pastor et al., 2015; Pavlov & Kotchoubey, 2019; Seligowski et al., 2018; Stolz et al., 2019). Modulations of LPP amplitudes through fear-conditioned stimuli indicate the activation of widespread brain systems that are related to perceptual, motivational, and motor signals (Wieser & Keil, 2020).

The event-related EEG waveform appears as a series of positive (e.g., P1, LPP) and negative (e.g., N170) peaks on the scalp (Kappenman & Luck, 2012). ERP components are typically defined with regard to their latency (time in milliseconds after stimulus onset), polarity (positive- or negative-going deflection), amplitude (change in voltage), and topography (spatial distribution on the scalp). Depending on the underlying ERP components (Miskovic & Keil, 2012; Wieser & Keil, 2020), elevated ERP amplitudes for CS+ compared with CS- can be either associated with a larger positive (for positive-going components) or negative (for negative-going components) deflection. Regarding the C1 component, which is not labeled with "P" ("positive") or "N" ("negative"), it is more complicated to predict the direction of this effect. Because of the retinotopic organization of the striate cortex, which is the assumed generator of the C1 component (Jeffreys & Axford, 1972; Rauss et al., 2011), this early ERP component can either appear as a negativity or positivity, and the polarity depends on whether the stimulus was shown in the upper or lower visual field, respectively (Clark et al., 1994). Figure 8 illustrates how fear-conditioned stimuli can evoke a more negative C1 amplitude when the C1 appears as a negative voltage deflection. It is important to keep in mind that the opposite effect (i.e., a more positive amplitude

for CS+) could also be possible. Furthermore, Figure 8 shows how CS+ can be associated with a more positive P1 amplitude. Under the assumption that the fear-conditioned CS+ requires more attention, one would expect that the P1 amplitude for the CS+ (compared with the CS-) should be more positive (Muench et al., 2016). However, the response pattern seems to be more complex. For example, Liu, Keil, & Ding (2012) demonstrated *decreased* P1 amplitudes after CS+ compared with CS- for well-trained stimuli (i.e., after several acquisition trials). This observation would be consistent with the interpretation that less allocation of attentional resources is required because the US is fully predicted by the CS+ (Pearce & Hall, 1980). This example illustrates that the formulation of hypotheses regarding the modulation of ERP components is not always straightforward. As already noted above, the literature on the emotional modulation of the N1/N170 period is mixed (Hajcak et al., 2012; Hinojosa et al., 2015; Rellecke et al., 2013; Schindler & Bublatzky, 2020). "N170" is the ERP label that is used for N1 responses to faces or face-like stimuli, which evoke a particularly large negative-going amplitude during this time window (Rossion & Jacques, 2012). Faces have been used as CSs in many studies, including all manuscripts of the present thesis. Face stimuli are extraordinarily prepared for fear conditioning (Mazurski et al., 1996; Öhman & Dimberg, 1978), due to their significance in the evolutionary past (Kret & Gelder, 2012). Thus, we would expect a larger N1/N170 amplitude for fear-conditioned stimuli (Camfield et al., 2016; Levita et al., 2015), but empirical results are inconsistent (Stolarova et al., 2006; Stolz et al., 2019). Regarding the LPP period, it is easier

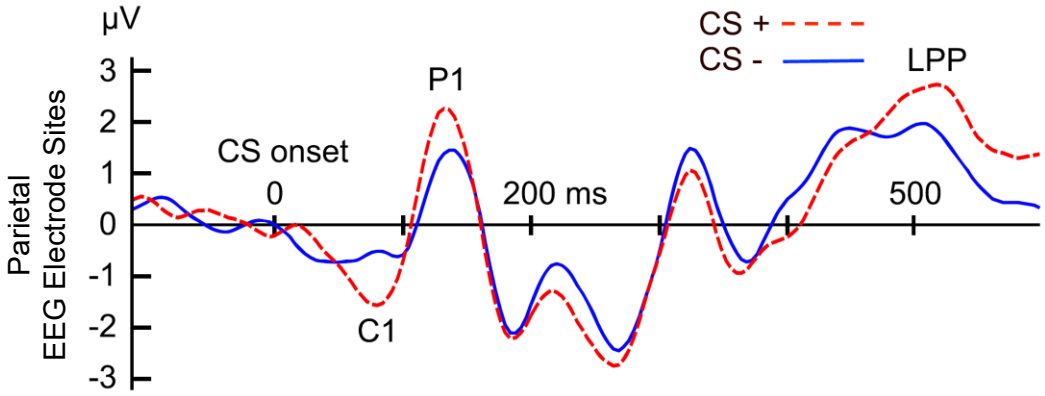


Figure 8. Illustration of differential event-related potential (ERP) responses to CS+ (dashed red line) and CS- (solid blue line) during fear acquisition. EEG data are taken form Stolarova et al. (2006). Amplitudes are enhanced for CS+ compared with CS- during the C1 period (more negative for CS+), during the P1 period (more positive for CS+), and during the LPP period (more positive for CS+). The CS+/- did not consist of face stimuli in this study, a factor that may explain the lack of ERP modulations during the N1/N170 period. This figure was originally published in a review of electrophysiological studies of human classical conditioning by Miskovic and Keil (2012). This figure is reprinted with permission from John Wiley & Sons, Inc. (Hoboken, NJ, USA) and from the Society for Psychophysiological Research (Madison, WI, USA). Permission was obtained through Copyright Clearance Center, Inc. (Danvers, MA, USA).

to derive a specific hypothesis, as CS+ versus CS- has been reliably associated with a larger positivity during late-latency time windows, as displayed in Figure 8 (for an overview of relevant studies, see Figure 7C). As highlighted above, EEG allows one to study neural correlates of fear conditioning with high temporal precision. A variety of studies have shown how fear-conditioned stimuli affect short-, mid-, and long-latency ERP components (see Figure 7C). Although several fear conditioning studies have applied EEG, two major limitations exist.

First, the EEG technique requires a massive number of trials to be averaged for an acceptable signal-to-noise ratio (Huffmeijer et al., 2014; Steinberg et al., 2013). Because of this methodological limitation, usually *all* (up to 60 or even more trials per CS condition) acquisition trials are averaged in typical EEG fear conditioning studies (Miskovic & Keil, 2012). This procedure ensures an adequate signal-to-noise ratio, but it can be highly problematic with regard to the validity of the experimental design. It has been argued in chapter 1.1 of the present thesis (see Figure 3) that conditioning theories assume a gradual *increase* in the conditioned fear response from early to late acquisition trials (Rescorla & Wagner, 1972; Tzovara et al., 2018). In other words: We would expect no (or at least a relatively small) conditioned fear response during early acquisition trials. The conditioned response should then rise during the subsequent trials, and a large conditioned response would only be expected during the last acquisition trials. However, restricting the EEG analyses to these final trials is not possible, as the signal-to-noise ratio would be too low. Instead, all trials are averaged in typical EEG studies (Miskovic & Keil, 2012), and temporal dynamics of learning are entirely ignored. In the *Encyclopedia of the Sciences of Learning*, Pavlovian conditioning has been defined as "the *adjustments* [emphasis added] organisms make in response to observing the temporal relations among environmental or proprioceptive stimuli" (Gottlieb, 2012). These adjustments (i.e., changes over time), which seem to be the core aspect of conditioning, have been ignored in almost all fear conditioning studies with EEG, where a large number of trials have been averaged (Miskovic & Keil, 2012). This is problematic not only because of validity issues related to learning principles, but also because of biological processes. In chapters 1.2 and 1.3, it has been argued that both animal studies and human fMRI studies indicate that the amygdala plays a key role in fear conditioning. Furthermore, amygdala activation shows a rapid habituation pattern over time (see chapter 1.3). This habituation curve has not only been ignored in several fMRI studies (as outlined in chapter 1.3); it has been neglected in almost all EEG studies. It is well known that EEG and MEG have problems isolating signals from deep structures such as the amygdala (Tzovara et al., 2019), but, nevertheless, activity from the amygdala seems to contribute to the modulation of ERP components that have been associated with fear conditioning (Bunford et al., 2018; Levita et al., 2015; Vuilleumier, 2009).

Given the assumed key role of the amygdala for threat processing (see chapter 1.2), there is an absolute necessity for human EEG studies to acknowledge the habituation pattern of this brain region and related neural circuits. An aggregation across a high number of trials will wash out any transient neurobiological processes, such as signals from the amygdala.

Second, as illustrated in Figure 7, the latencies that have been investigated in previous human EEG studies are highly inconsistent. Some authors have focused on early ERP components (e.g., C1) but have ignored modulations of slower ERP waves. Other authors have investigated mid-latency components (e.g., P1/N1/N170). While the majority of studies have analyzed late-latency LPP responses, most of these LPP studies did not assess earlier ERP effects. If time windows for ERP analyses are restricted to specific components, other effects (e.g., effects that might occur during periods of low amplitude) can easily be missed (Murray et al., 2008). Furthermore, the exact time windows during which voltage changes are extracted for statistical analyses have often been based on "prior research" or "visual inspection" (Keil et al., 2014). These procedures can be highly problematic and lead to invalid conclusions. Previous studies may differ in filter characteristics (Acunzo et al., 2012; Duncan et al., 2009; Hajcak et al., 2012; Tanner et al., 2015, 2016) and stimulus luminance or discriminability (Luck & Gaspelin, 2017), which are known factors that can affect the visibility, latency, and duration of ERP effects. If relevant time windows are based on "visual inspection" of the data (e.g., of the aggregate grand average ERP; Brooks et al., 2017), other problems arise. Visual data inspection typically aims to identify clearly visible peaks in the ERP wave, and the mean voltage change surrounding a respective peak is further subjected to statistical analyses. However, although scalp peaks are often used for common ERP terminology (e.g., "P1 component" when referring to the first visible positive peak), it is important to keep in mind that visually salient peaks in the observed scalp ERP waveform are not necessarily equivalent to discrete intracranial sources (underlying "latent" ERP components) that are related to certain neurocognitive processes (Kappenman & Luck, 2012). It has been pointed out by Luck (2014) that there "is nothing special about the point [i.e., the peak] at which the voltage in the observed waveform reaches a local maximum." Instead, *underlying* components should be conceptualized as latent processes in the brain that sum together and, thereby, generate a visible ERP wave on the scalp, which can then be measured with EEG electrodes (Luck & Kappenman, 2019). Each visible peak in the scalp waveform is typically generated by several (i.e., more than one) underlying components in the brain (Kappenman & Luck, 2012; Luck, 2014). In neurophysiological research, we are typically interested in these underlying components, but – as EEG is a noninvasive technique – we have to rely on the scalp waveform. It is obvious that it is problematic when conclusions on latent components (representing discrete intracranial sources)

are derived from more or less "meaningless" (Luck, 2014) peaks. It is often ignored that a single peak in the waveform is actually generated by several "components" (indicating intracranial sources). Furthermore, the current labeling (e.g., "P1 component") is rather misleading. This problem has been elaborated by Kappenman and Luck (2012, p. 4): "The term *ERP component* is more challenging to define. This term gets bandied about in the literature very frequently, but it is rarely defined or conceptualized beyond the peaks in the observed ERP waveform."

Returning to the field of fear conditioning, the current practice of selecting time windows for EEG analyses has several methodological shortcomings. This is even true for the LPP period, although LPP modulations seem to be among the most robust ERP fear conditioning effects (see Figure 7C). In the majority of studies, researchers have restricted the analysis window to periods between 300 and 800 ms (e.g., Bacigalupo & Luck, 2018; Ferreira de Sá et al., 2019; Pastor et al., 2015; Pavlov & Kotchoubey, 2019), but the exact latencies vary enormously among studies. Sometimes, LPP is analyzed during relatively early periods, starting already around 250 ms (e.g., Panitz et al., 2015). Other authors have extended the analysis to later (e.g., 1,200 ms; Seligowski et al., 2018) or even very late (e.g., 2,000 ms; Stolz et al., 2019) periods. These problems turn the selection of appropriate time windows into a somewhat speculative endeavor (Clayson et al., 2019; Michel & Murray, 2012; Miskovic & Keil, 2012), which has already been pointed out by Pizzagalli et al. (2003, p. 185):

> "Although several ERP studies have investigated cortical correlates of classical aversive conditioning, results often diverge. Whereas some studies have demonstrated CS+ modulation during acquisition of fear conditioning on early ERP components (P100, N100, P200) […], others have found modulations of later ERP components, particularly the P300 […] and the CNV complex […]."
>
> (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*, 184–194.)

In a similar way, Ferreira de Sá et al. (2019, p. 190) emphasized:

> "Electro-cortical evidence regarding threat learning and extinction is, however, still scarce and inconsistent […]. Although there seems to be a consensus on the fact that differential CS processing can be seen in electro-cortical responses in general, the exact timing and topographical properties of these effects and their respective functional meaning remain unclear. […] One reason for the diversity of the reported effects may be the fact that they are likely to comprise a multitude of different neurocognitive processes involved in fear learning and extinction."
>
> (Ferreira de Sá, D. S., Michael, T., Wilhelm, F. H., & Peyk, P., 2019, Learning to see the threat: Temporal dynamics of ERPs of motivated attention in fear conditioning. *Social Cognitive and Affective Neuroscience*, *14*, 189–203.)

Steinberg et al. (2013) complained that especially early ERP modulations have often been overlooked in fear conditioning studies and concluded that "there is compelling evidence showing that the speed of cortical stimulus processing should be revised" (Steinberg et al., 2013).

Taken together, two problems arise from previous EEG fear conditioning studies. First, regarding the temporal dynamics of threat learning, it has been widely ignored how conditioned electrocortical responses *increase* during fear acquisition and *decrease* during fear extinction, respectively. Second, there is an enormous variance in the fear conditioning literature with regard to investigated ERP components (e.g., C1, P1, N170, LPP) and exact time windows that are used for statistical inference. The selection of appropriate time windows for ERP analyses is challenging and often speculative, especially for new paradigms. In manuscript 3 of the present thesis (Sperl et al., 2021), we have addressed both issues. On the one hand, we developed a new sequential-set fear conditioning paradigm that allows tapping into more habituation-prone neural processes and helps to unravel the learning curve of ERP correlates throughout fear acquisition and extinction. On the other hand, we circumvented the problem of selecting appropriate time windows for statistical analyses. Instead, we applied data-driven topographic EEG analyses to identify periods with significant map differences between conditions. Importantly, this approach does not make any *a priori* assumptions concerning electrode sites or time windows.

The previous paragraphs have described how the ERP technique can be used to obtain fine-grained information regarding the time course of neural processes involved in fear conditioning. However, the traditional ERP technique is not the only way to analyze EEG signals. Indeed, *oscillatory EEG activity* describes another measure of event-related EEG activity and can provide further information beyond the traditional ERP method (Bastiaansen et al., 2012). Event-related EEG activity can be analyzed not only in the time domain, but also in the frequency domain. Cohen (2014) pointed out that traditional ERP analyses reveal only a certain part of the information that can be derived from the EEG data. Important task-related information can be overlooked after ERP averaging across epochs (Cohen, 2014). It has already been described above that, to achieve an adequate signal-to-noise ratio, EEG analyses require a large number of trials to be averaged (Huffmeijer et al., 2014; Steinberg et al., 2013). In fear conditioning studies, for example, EEG segments time-locked to the CSs are averaged (Miskovic & Keil, 2012). This approach reduces noise in the signal, as noise is assumed to be distributed randomly across trials of a single CS type. Traditional ERP analyses are interested in *time-* and *phase-locked* activity that is initially (e.g., before the CS onset) "nonexistent" and *evoked* by the event (the CS in fear conditioning studies). However, this approach ignores that EEG oscillations are ongoing and exist even in the absence of the experimental task. At the time of the stimulus onset, the phase may thus be variable. Non-

phase-locked EEG responses are canceled out through the traditional ERP averaging method. Event-related analyses of oscillatory responses can be understood as modulations of *ongoing* EEG activity, and we are thus also interested in *non-phase-locked* responses (Bastiaansen et al., 2012). This major difference between traditional ERP analyses and the study of oscillatory dynamics can be illustrated by means of an important difference in the EEG processing stream. For traditional ERP analyses, the EEG activity is first averaged across several trials, and the mean voltage change (e.g., during the LPP period) is then extracted from the *averaged* ERP. It is obvious that any non-phase-locked responses will be missed. By contrast, when we are interested in event-related oscillatory responses, the order of analytical steps is different. Frequency-based analyses (e.g., Fast Fourier Transform) are first applied for each single trial (e.g., each CS epoch), and the estimated single-trial power (i.e., squared amplitude) is *then* averaged across trials. The major advantage of frequency-based EEG analyses is that results can be better interpreted with regard to neurophysiological mechanisms, and this ability allows researchers to explore synchronization and desynchronization patterns of neuronal activity (Cohen, 2014). Non-phase-locked responses can be considered a measure that predominantly reflects to which extent the underlying neuronal populations synchronize (Donner & Siegel, 2011; Pfurtscheller & Lopes da Silva, 1999). Synchronous oscillations seem to play a key role in linking different brain areas of a functional network (Bastiaansen et al., 2012; Klimesch, 1996; Lopes da Silva, 2013).

In chapter 1.2, the "traditional" neurobiological model of threat processing has been introduced to highlight the prominent role of AMC-amygdala connectivity during fear expression (see Figure 4). Rodent research indicates that theta oscillations from the PL (the assumed rodent homolog of the human AMC; Milad & Quirk, 2012) seem to be relevant for sustained fear processing (Burgos-Robles et al., 2009; Narayanan et al., 2011; Pitman et al., 2012), and theta synchrony may play a key role for bidirectional connectivity between the PL/AMC and amygdala (Courtin et al., 2014; Gilmartin et al., 2014; Senn et al., 2014). These findings have been extended in non-human primates, showing that fear conditioning increases amygdala-AMC theta synchrony (Taub et al., 2018). Translating these findings into humans, Mueller et al. (2014) applied 64-channel EEG recordings to demonstrate that healthy participants show enhanced theta oscillations (4–8 Hz) at frontomedial EEG channels when previously fear-conditioned and nonextinguished stimuli are shown. Theta power was further source-localized to the AMC (Mueller et al., 2014), supporting the assumed role of theta oscillations in AMC-amygdala connectivity (Gilmartin et al., 2014). However, Mueller et al. (2014) could not assess explicitly amygdalar activity, due to limitations of the EEG method to isolate signal from subcortical structures (Tzovara et al., 2019). In manuscript 6 of the present thesis (Sperl et al., 2019), we aimed to close this gap. Specifically, we

applied simultaneous EEG-fMRI measurements in a human fear conditioning paradigm to explore the expected interplay between frontomedial theta power (EEG) and subcortical amygdala activation (fMRI).

1.5 Brain Responses Trigger Changes in Peripheral Physiology: Peripheral Physiological Correlates of Human Fear Conditioning

Peripheral physiological measures, especially indicators of autonomic nervous system activity, have long been key dependent variables in human fear conditioning experiments (Lipp, 2006). These measures have high ecological validity, given that many patients with fear-related disorders experience similar peripheral symptomatology (e.g., sweating, changes in heart rate) when confronted with a feared object or situation (Craske et al., 2017; Craske & Stein, 2016; Stein et al., 2019; Yehuda et al., 2015). A major advantage of these peripheral measures (e.g., compared with EEG) is that they have a relatively good signal-to-noise ratio and can thus better capture changes during learning (Lipp, 2006).

At the beginning of this thesis, the "two-system" framework by LeDoux and colleagues (LeDoux, 2017; LeDoux & Daw, 2018; LeDoux & Pine, 2016) has been explained (see Figure 1). This framework suggests that threat activates a "defensive survival circuit" in the brain, which further generates *defensive behavior* and *changes in peripheral physiology*. Besides brain substrates, in most fear conditioning studies (including all manuscripts that are part of this thesis) researchers also investigate peripheral physiological responses. The study of peripheral correlates during threat processing helps to understand and to interpret better the functional relevance of observed changes in neurophysiology (e.g., "fight-or-flight" behavior; Davis & Lang, 2003; LaBar, 2018). Furthermore, measures of peripheral physiology allow one to bridge between human research and animal studies, which also often include peripheral indices of threat (e.g., heart rate changes and freezing; Headley & Weinberger, 2013; Roelofs, 2017).

Electrodermal activity is the peripheral measure that is most frequently used in human fear conditioning studies (Lonsdorf et al., 2017; Marin et al., 2017), although some individuals show an absence of reactivity in skin conductance (Marin et al., 2020). Fear acquisition is typically associated with elevated SCRs for CS+ compared with CS- (Lipp, 2006; Lonsdorf et al., 2017), as illustrated in Figure 9A. During fear extinction, this differentiation shows a gradual decrease (Sperl et al., 2021). Elevated SCRs can be interpreted as a marker of sympathetic activation (Bach et al., 2009; Boucsein, 2012; Choy et al., 2015) and enhanced arousal (Bach et al., 2009; Bach et al., 2010; Boucsein et al., 2012; Critchley, 2002), due to orientation to the salient CS+ and anticipation of the US (Lipp, 2006; Prokasy, 1977; Prokasy & Kumpfer, 1973). Elevated sympathetic arousal has been associated with "fight-or-flight" behavior (Critchley, 2002; Davis & Lang, 2003) in light of the threatening nature of the CS+ and US.

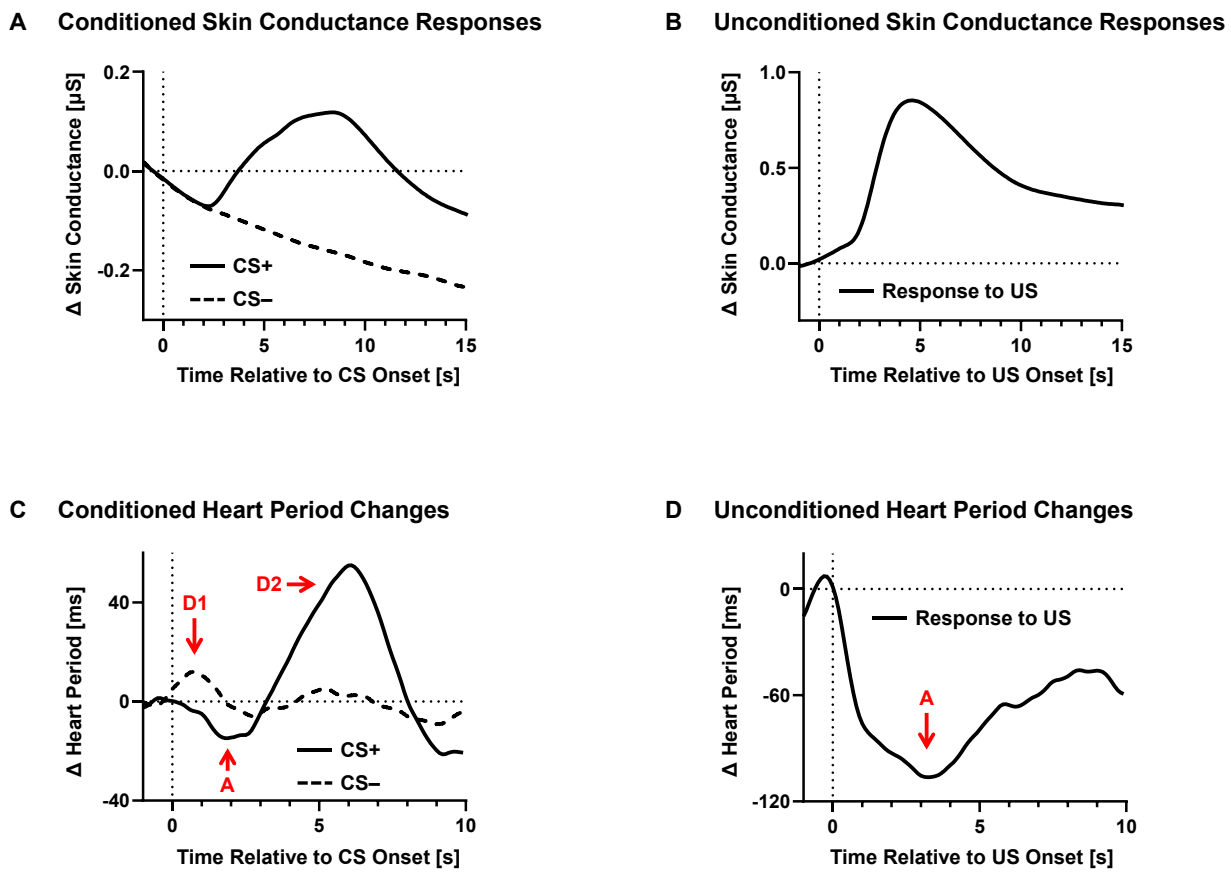


Figure 9. Peripheral physiological correlates of fear acquisition. After averaging across several trials, (A) the CS+ (compared with the CS-) and (B) the US are associated with elevated skin conductance responses (SCRs). Furthermore, (C) CS+ (compared with CS-) evoke cardiac deceleration ("D2" written in red), while (D) the US is followed by cardiac acceleration ("A" written in red). Responses to the CS show a three-phasic response pattern, starting with a brief deceleration ("D1" component), which is followed by acceleration ("A" component) and deceleration ("D2" component) components (as indicated in red color). Fear-acquisition is consistently associated with a larger D2 component for CS+ versus CS-. The data used for illustration in this figure are taken from a study by Sperl et al. (2021). This study is described in more detail in manuscript 3 of this thesis.

In addition to electrodermal activity, several studies have assessed heart rate changes as a measure of conditioned fear or threat (Bradley et al., 2005; Castegnetti et al., 2016; Lonsdorf et al., 2017). In human fear conditioning experiments, heart rate responses to the CSs usually follow a three-phasic response pattern (Lipp, 2006). As can be seen in Figure 9C, an initial cardiac deceleration (D1) is followed by a transient acceleration (A) and a large second deceleration (D2) component. Several studies have shown that fear acquisition consistently evokes a larger second deceleration (D2) for CS+ versus CS- (Deane & Zeaman, 1958; Gruss et al., 2016; Notterman et al., 1952; Panitz et al., 2015; Schipper et al., 2019; Thigpen et al., 2017; Yin et al., 2018), and differential cardiac responses diminish throughout fear extinction (Sperl et al., 2021). Fear-conditioned bradycardia has been interpreted as heightened vigilance to the CS+, due to

anticipation of the dangerous US (Davis & Lang, 2003; Löw et al., 2015). From an evolutionary perspective, facilitated sensory intake and the allocation of attentional resources ("attentive freezing") can be crucial for survival (Blanchard et al., 2011; Lang & Bradley, 2010; Mobbs et al., 2015; Roelofs, 2017). As shown in Figure 9C, cardiac deceleration is typically indicated as a change in interbeat intervals (IBIs). Note that there are two measures that are commonly used for cardiac activity (Jennings et al., 1981): heart *rate* (the number of beats per minute) and heart *period* (i.e., IBI, the time between two R waves in milliseconds). Both measures are reciprocal quantifications of the electrocardiogram and can easily be converted. At first glance, it looks as though the selection of one of these cardiac metrics (heart rate or heart period) is not important. However, both measures are not linearly related (Berntson et al., 2019). Stern et al. (2001) explained in greater detail that there is a relatively linear relationship between heart period and (para-)sympathetic stimulation. However, if heart period is transformed to heart rate, a statistical artifact is introduced, and the linear relationship with (para-)sympathetic change is distorted (Berntson et al., 2019; Stern et al., 2001). According to a recommendation of Berntson et al. (1995), heart period (versus heart rate) should be used when changes in cardiac function seem to be related to autonomic effects, which is of particular relevance for psychophysiological studies (Stern et al., 2001). In all manuscripts of the present thesis that used electrocardiographic data, we used *heart period* (i.e., IBIs) to quantify cardiac responses.

Besides electrodermal and cardiac methods, authors of some fear conditioning studies have applied the fear-potentiated startle technique (Blumenthal et al., 2005). Assessing threat-related modulations of human eyeblink responses extends knowledge from animal research, where physiological mechanisms of the startle reflex are well understood (Daldrup et al., 2015; Falls, 2002; Lang et al., 2000). In psychophysiological studies, the startle reflex is typically elicited by acoustic white noise bursts (see Figure 10A) and quantified with electromyographic (EMG) recording electrodes (Blumenthal et al., 2005). In anticipation of the aversive US, several studies have shown that the startle reflex is potentiated during CS+ (compared with CS-) presentations (e.g., Khemka et al., 2017; Lindner et al., 2015; Norrholm et al., 2011; Seligowski et al., 2018; Soeter & Kindt, 2011, 2012b), which is illustrated in Figure 10B. In an evolutionary context, the startle reflex seems to promote survival in life-threatening situations (Lang et al., 2000). Blink startle is a reflexive reaction primarily mediated by brainstem structures (Lipp, 2006). Based on this knowledge, fear-potentiated startle is a physiological method representing processes that seem to be rather outside of conscious control (Hamm & Weike, 2005; Oyarzún et al., 2019; Sevenster et al., 2014; Weike et al., 2007). Referring to the "two-system" framework by LeDoux and colleagues (LeDoux, 2017; LeDoux & Daw, 2018; LeDoux & Pine, 2016), fear-potentiated startle

measures are thus related to defensive survival circuits in the brain (see Figure 1). This conceptualization is in line with findings that amygdalar lesions block fear-potentiated startle responses (Hamm & Weike, 2005). Given that the "two-system" framework states that the mental state of "fear" is particularly related to cognitive circuits (in contrast to subcortical defensive survival circuits, see Figure 1), the term "fear-potentiated startle" may be misleading. In light of recent discussions about precise terminology (Adolphs, 2013; Fanselow & Pennington, 2017; LeDoux, 2014; LeDoux & Daw, 2018; LeDoux & Hofmann, 2018; Mobbs, 2018), a more descriptive term (e.g., "threat-potentiated startle") might be more appropriate, although "fear-potentiated startle" is still commonly used in the literature (Lonsdorf et al., 2017).

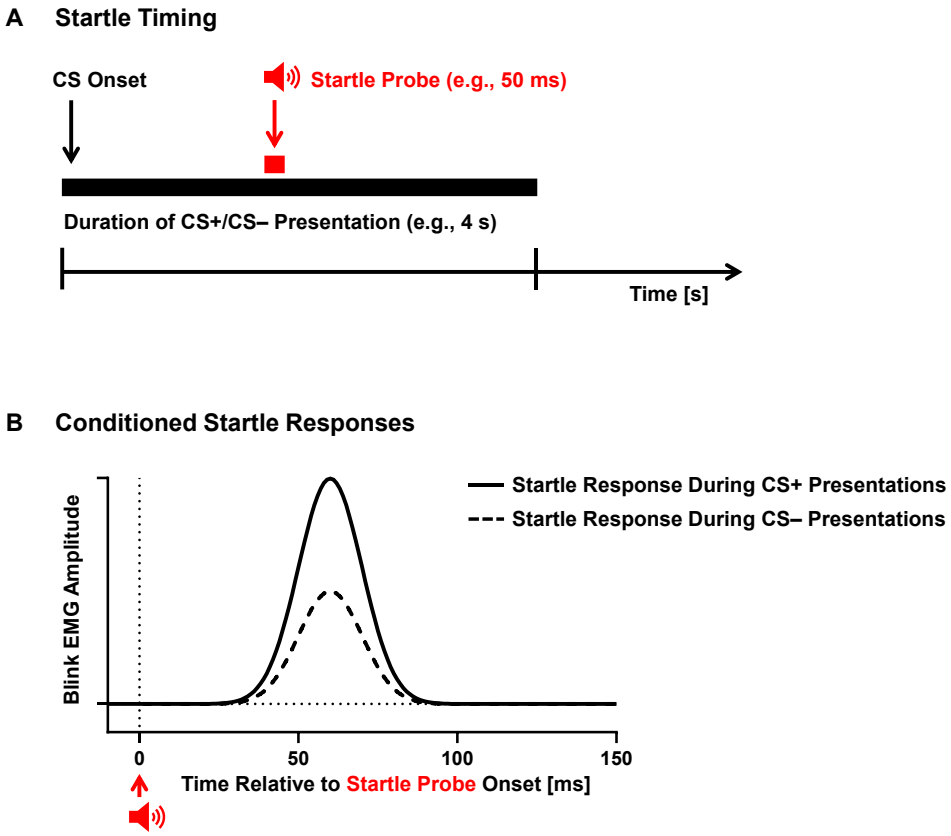


Figure 10. Illustration of the fear-potentiated startle response. (A) Startle probes (e.g., acoustic white noise bursts) are presented during a time window that overlaps with the presentation of the CSs. The latency of the startle probe onset (relative to the CS onset) can vary between trials. (B) During fear acquisition, startle probes that are presented during CS+ (compared with CS-) evoke enhanced startle blink responses. Blinks are usually measured using an electromyogram (EMG), with electrodes placed over the orbicularis oculi muscle (i.e., the muscle that closes the eye during a blink). Note that EMG responses are time-locked to the onset of the startle probe (e.g., loud noise, indicated in red color), *not* to the CS onset (as for skin conductance responses and heart rate changes). During preprocessing, the EMG signal is rectified. In previous studies, authors have used different measurement units to report eyeblink EMG amplitude (e.g., analog-to-digital units, arbitrary units, microvolts, or, for integrated EMG, microvolt · seconds). The visualization in panel B has been created for illustrative purposes and is based on simulated data. To prevent invalid conclusions, the range of the *y*-axis is not specified further. The visualization is based on previous illustrations (Blumenthal et al., 2005; Leuchs et al., 2019; Lonsdorf et al., 2017; Newman et al., 2010).

Some authors did not restrict peripheral physiological analyses on conditioned responses (i.e., responses to the CS), but they were also interested in unconditioned responses (i.e., responses to the US). The US consistently evokes elevated SCRs (see Figure 9B; Dunsmoor et al., 2008; Goodman et al., 2018; Knight et al., 2010, 2011) and cardiac acceleration (see Figure 9D; Ginsberg & Thysell, 1966; Lipp, 2006; Lipp & Vaitl, 1990; Vila et al., 2007). It has often been ignored that conditioned and unconditioned responses can be qualitatively different, and, for cardiac responses, can even have opposite polarity (deceleration versus acceleration). In manuscript 3 of the present thesis (Sperl et al., 2021), we demonstrate that cardiac responses as well as EEG signatures are qualitatively different for conditioned (i.e., evoked by CS+ versus CS-) compared with unconditioned (i.e., evoked by the US) responses. This discrepancy, which has often not been considered adequately, has important implications for theories and conceptualizations about fear conditioning. This issue is further addressed in chapter 3.4 (discussion section).

A major limitation of peripheral physiological measures is that they are sensitive but not specific for *fear* or aversive stimulus processing. It has been shown that highly arousing appetitive stimuli (e.g., visual sexual stimuli) also evoke elevated SCRs (Costa & Esteves, 2008) and modulate heart rate deceleration/acceleration (Bradley et al., 2001). Appetitive conditioning elevates peripheral physiological responses in a way that resembles fear conditioning (Andreatta & Pauli, 2015; Klucken et al., 2015; Kruse et al., 2017; Tapia León et al., 2018; but: Both et al., 2008; Hoffmann et al., 2004; Klucken et al., 2009). This limitation does not only apply to peripheral measures. Similarly, fMRI studies have shown that appetitive conditioning recruits the amygdala (Klucken et al., 2009; Klucken et al., 2015; Klucken, Wehrum-Osinsky et al., 2016; Kruse et al., 2017; Schweckendiek et al., 2016), a brain region that plays a pivotal role in neurobiological models of fear and threat (see chapter 1.2). The nonspecificity of physiological measures for negative affective states is critical, especially in light of recent discussions about precise terminology of “fear”-related processes in neuroscience (Adolphs, 2013; Fanselow & Pennington, 2017; LeDoux, 2014; LeDoux & Daw, 2018; LeDoux & Hofmann, 2018; Mobbs, 2018), which culminated in the development of the “two-system” framework (see Figure 1) of threat processing (LeDoux, 2017; LeDoux & Daw, 2018; LeDoux & Pine, 2016).

To gain a better and more specific understanding of *mental states* (e.g., the subjective experience of “fear”), all studies that are included in this thesis also applied *subjective ratings* (i.e., CS-associated valence, arousal, and fear, see chapter 1.2). Combining different methods provides a more complete picture of the conditioned response.

1.6 Catecholaminergic Mechanisms of Fear Conditioning: Making Translation Work?

In the previous chapters, fear conditioning has been explained as a learning model that describes how "fear" is acquired in animals and humans. It has also been clarified that the term "fear" can be misleading, especially when evaluating results from animal models (LeDoux, 2017; LeDoux & Daw, 2018; LeDoux & Pine, 2016). Nevertheless, the term "fear conditioning" is commonly used in research and in conditioning-based applications (e.g., exposure therapy). Therefore "fear" conditioning (rather than "threat" conditioning, which would be more precise; LeDoux, 2014) is used continuously throughout this thesis. However, the constraints and potential pitfalls of this term (which have been described in the previous chapters) should be kept in mind, especially when translating findings from animal studies to human applications (Haaker et al., 2019; Lonsdorf et al., 2017).

Fear extinction is considered to be the most prominent learning model underlying highly efficient exposure therapy interventions in fear-related disorders (Craske et al., 2018). During exposure therapy, patients are repeatedly confronted with the feared stimulus, while the feared outcome does not occur (Craske et al., 2014). For example, pathological fear in patients suffering from spider phobia can be reduced by repeated confrontations with a live tarantula (Hauner et al., 2012) or with images of spiders (Watson et al., 2019). The neurobiological model of threat processing, which has been described in more detail in chapter 1.2 (see Figure 4), suggests an inhibitory memory trace that is established during exposure. Precisely, activation of the vmPFC is thought to inhibit amygdala-mediated fear expression (Milad & Quirk, 2012).

Fear conditioning and extinction mechanisms describe how fear is learned and "unlearned," respectively. However, several questions remain open. From an evolutionary perspective, fear learning has several adaptive functions to increase the probability of survival when confronted with danger (Adolphs, 2013; Öhman, 2009). But it is important to consider: Why can fear conditioning – which is an adaptive learning mechanism – contribute to the etiology and maintenance of anxiety disorders in some people? Fear extinction seems to be evolutionary adaptive as well, as it allows one to adjust behavior to changing environments when stimuli no longer predict danger (Delgado et al., 2006). Exposure therapy is highly effective (Craske et al., 2017; McNally, 2007). Remarkably, only a single exposure session can lead to striking improvement for patients with a specific phobia (Öst et al., 1992; Öst et al., 2001). Nevertheless, not all patients benefit sufficiently, and researchers need to explore how standard procedures can be optimized to reach improvement in those patient subgroups as well (Hofmann et al., 2011; Lebois et al., 2019; Weisman & Rodebaugh, 2018). Neuroscientific methods should be used to

understand (a) why some people (but not others) acquire *pathological* fear, and (b) how pathological can be better *reduced* when state-of-the-art procedures (i.e., standard exposure therapy) are not sufficient. The study of the catecholaminergic system is very promising to generate innovative answers to these questions.

The catecholaminergic neurotransmitters dopamine and noradrenaline (norepinephrine) are of particular interest for the present thesis. Dopamine and noradrenaline are synthesized from the amino acid tyrosine and L-DOPA (Eisenhofer et al., 2004), as shown in Figure 11. The vesicular monoamine transporter 2 (VMAT2) transports dopamine into synaptic vesicles (Eiden et al., 2004), where – in the case of dopaminergic neurons – dopamine is accumulated until its release into the extracellular space. In noradrenergic neurons, the membrane of these vesicles contains the enzyme dopamine β-hydroxylase (DBH), which synthesizes noradrenaline from dopamine (Rush & Geffen, 1980). Catechol-*O*-methyltransferase (COMT) is an enzyme that is involved in degrading (Eisenhofer et al., 2004; Ho & Weinshilboum, 2019) extracellular catecholamines (see Figure 11). There are also other breakdown mechanisms, but COMT is of particular interest for the present thesis and is therefore explicitly included in Figure 11.

Dopamine and noradrenaline are neurotransmitters that are well known to modulate the consolidation of emotional memories (LaLumiere et al., 2017; McGaugh, 2013, 2015; O'Donnell et al., 2004). In two studies of the present thesis, we aimed to explore catecholaminergic modulations of conditioned and extinguished fear (see Figure 11). On the one hand, abnormal catecholaminergic activity (e.g., due to elevated noradrenergic arousal in the aftermath of a traumatic event) may play a crucial role in the etiology of pathological fear (Kapfhammer, 2013). Specifically, heightened catecholaminergic transmission may strengthen the consolidation of conditioned fear. Thus, learning mechanisms that would be adaptive under "normal" circumstances could become maladaptive under altered catecholaminergic activity. On the other hand, catecholamines may play a pivotal role for improving the effectiveness of exposure therapy. Pharmacological substances that alter catecholaminergic activity may explicitly be used to boost the consolidation of extinction memories after exposure therapy (Bowers & Ressler, 2015; Dunlop et al., 2015; Holmes & Quirk, 2010). Both mechanisms (i.e., actions on consolidation of *conditioned* and *extinguished* fear) are illustrated in Figure 11.

The present thesis includes two manuscripts (manuscript 4: Sperl et al., submitted; manuscript 5: Panitz, Sperl et al., 2018) in which we investigated catecholaminergic mechanisms of fear conditioning and extinction. In particular, we used two scientific approaches, which are marked with red arrows in Figure 11. In manuscript 4 (Sperl et al., submitted), we applied dopaminergic

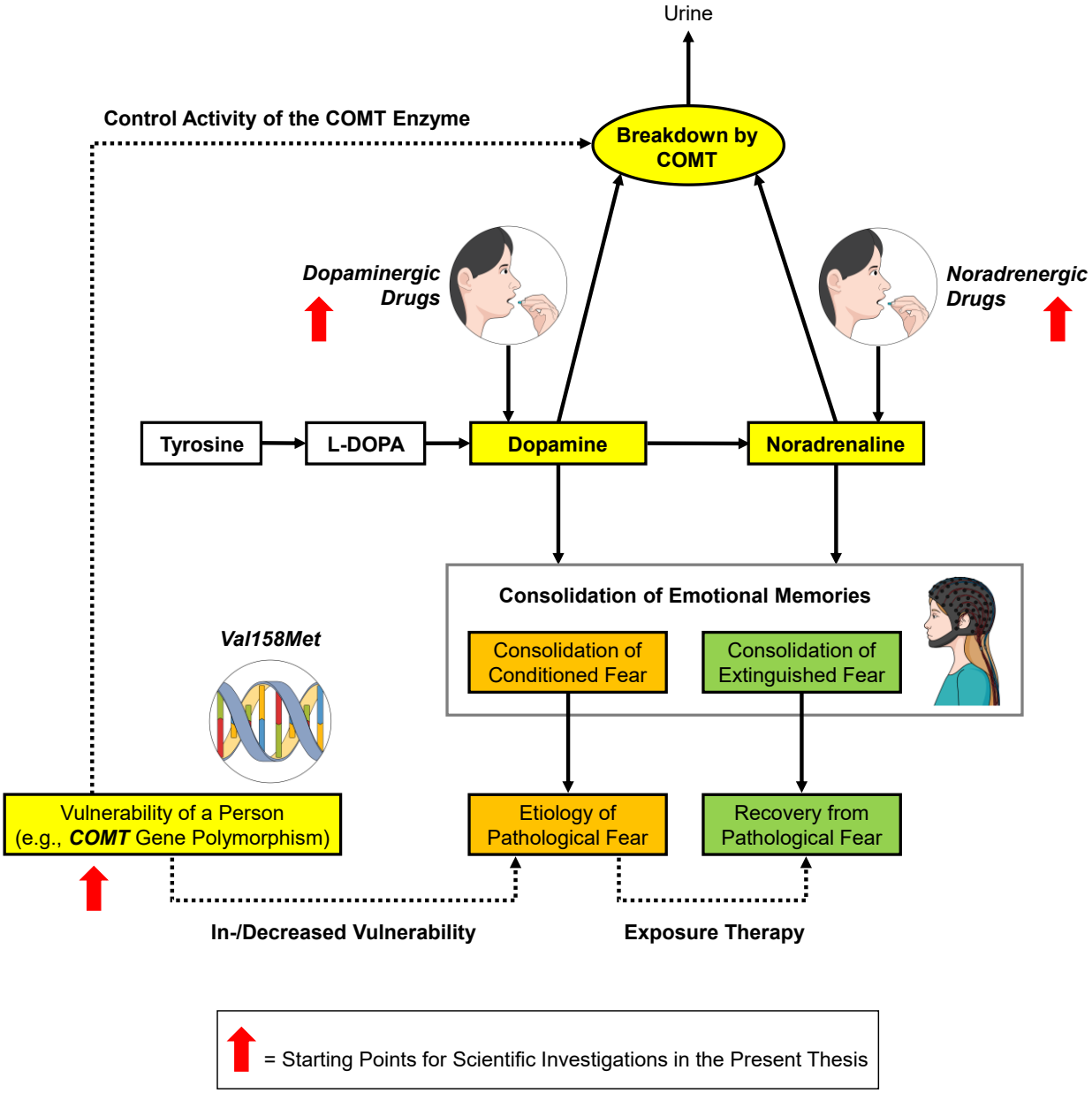


Figure 11. The catecholamines dopamine and noradrenaline are synthesized from tyrosine and L-DOPA. Dopamine and noradrenaline play critical roles in the consolidation of emotional memories. Both neurotransmitters have been linked to consolidation mechanisms in the context of fear conditioning and extinction. On the one hand, catecholaminergic modulation of conditioned fear contributes to a neurobiological model that could explain how overconsolidation processes are involved in the etiology of pathological fear (e.g., specific phobias or post-traumatic stress disorder). On the other hand, successful consolidation of extinguished memories (which also seems to be affected by catecholamines) is essential for the effectiveness of exposure therapy. Catechol-*O*-methyltransferase (COMT) is an enzyme that is involved in the breakdown of dopamine and noradrenaline. The vulnerability of an individual to develop fear-related disorders may be partially linked to polymorphisms of the *COMT* gene. Specifically, the Val158Met polymorphism is associated with COMT-dependent degradation of dopamine and noradrenaline. This mechanism may explain why individuals with certain genotypes could be more vulnerable to elevated fear acquisition or impaired fear extinction. The red arrows illustrate starting points for scientific investigations of the present thesis. In manuscript 4 (Sperl et al., submitted), we investigated the effects of dopaminergic or noradrenergic substances on the consolidation of conditioned and extinguished fear. In manuscript 5 (Panitz, Sperl et al., 2018), we explored associations between *COMT* Val158Met genotype and fear conditioning/extinction.

and noradrenergic drugs to manipulate catecholaminergic activity experimentally. To assess catecholaminergic effects on fear conditioning, physiological correlates (especially EEG) of threat processing were measured. In manuscript 5 (Panitz, Sperl et al., 2018), we chose a different approach. Instead of explicitly manipulating catecholaminergic activity, we investigated the Val158Met polymorphism of the human *COMT* gene, which is associated with COMT-dependent degradation of dopamine and noradrenaline (Lachman et al., 1996; Lotta et al., 1995). Both scientific approaches are outlined in more detail below.

The noradrenergic drug yohimbine has attracted great interest in neuroscientific fear conditioning research (Giustino & Maren, 2018; Holmes & Quirk, 2010; LaLumiere et al., 2017). Yohimbine is an indole alkaloid that promotes central and peripheral release of noradrenaline (Goldberg & Robertson, 1983). In the brain, the locus coeruleus is the principal site for noradrenaline synthesis (Schwarz & Luo, 2015). Yohimbine increases locus coeruleus firing and stimulates the release of noradrenaline, as it acts as an antagonist at presynaptic α2 autoreceptors (Dunlop et al., 2012, 2015; Singewald et al., 2015). This mechanism leads to elevated activation of postsynaptic β1 receptors, which seem to be critical for yohimbine's effects on fear and extinction consolidation (Dunlop et al., 2015). The mechanism of yohimbine on noradrenergic synaptic activity is illustrated in Figure 12.

Previous studies have shown that yohimbine can (a) strengthen fear consolidation (which might explain why noradrenaline could play a pivotal role in the etiology of fear-related disorders) and (b) boost extinction learning (suggesting that noradrenergic stimulation could facilitate exposure therapy). Rodent research has elucidated that administration of the noradrenergic drug yohimbine facilitates fear consolidation (Gazarini et al., 2013) and can lead to a PTSD-like fear memory in animals (Davis et al., 1979; Gazarini et al., 2014). These findings suggest that yohimbine might be suited to experimentally model states of pathological fear. Human studies are in line with the proposed hyperconsolidation concept in PTSD (Nicholson et al., 2014), demonstrating that yohimbine administration strengthens the consolidation of fear-conditioned startle responses (Soeter & Kindt, 2011, 2012b).

Memories about events that are associated with high emotional arousal are better consolidated (LaLumiere et al., 2017; Mather et al., 2016; McGaugh, 2015). As displayed in Figure 13, this process involves brain areas that are also involved in threat processing (e.g., the amygdala and several cortical areas). These brain networks have already been described in more detail in chapter 1.2 (see also Figure 4). Figure 13 is an extension of Figure 4 and illustrates how emotional arousal could modulate brain regions that play key roles in threat processing. Emotionally arousing events

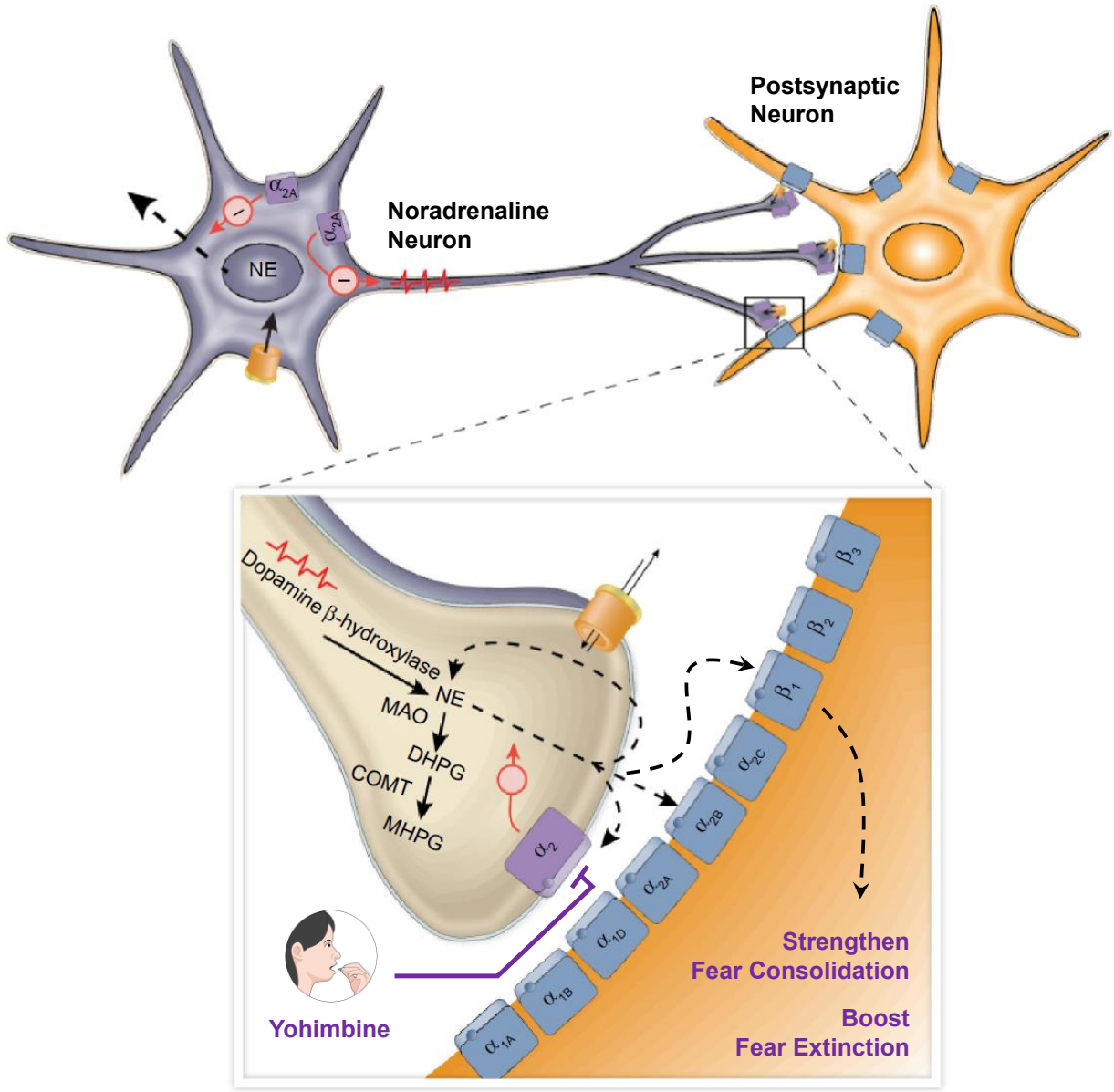


Figure 12. Illustration of yohimbine actions on noradrenergic synaptic activity (Montoya et al., 2016). Yohimbine acts as an antagonist at presynaptic α2 autoreceptors. The blockade of inhibitory autoreceptors leads to elevated noradrenergic release. The subsequent activation of postsynaptic β1 receptors seems to be critical for yohimbine's effects on fear and extinction consolidation (Dunlop et al., 2015). The catabolic enzymes monoamine oxidase (MAO) and catechol-*O*-methyltransferase (COMT) are involved in the degradation of noradrenaline. DHPG = dihydroxyphenylglycol; MHPG = 3-methoxy-4-hydroxyphenylglycol. This figure was originally published in a review by Montoya et al. (2016). For the present thesis, this figure was adapted to illustrate pharmacological actions of yohimbine. The figure is reprinted with permission from Taylor & Francis (Abingdon, UK).

activate the noradrenergic system, and noradrenergic release seems to play a pivotal role for the memory-enhancing effects (LaLumiere et al., 2017; Mather et al., 2016; McGaugh, 2013). Note that these effects are related to the intensity of arousal, irrespective of associated valence

(McGaugh, 2013). Highly arousing events can be positive (e.g., a person's wedding or birthday parties) or negative (traumatic events like a car accident, a mugging, or sexual abuse). All of these events seem to be associated with heightened noradrenergic arousal (LaLumiere et al., 2017). A prominent example is related to the terrorist attack on September 11, 2001 in New York City (Sharot et al., 2007). Compared with people staying a few miles away, those individuals who were in downtown Manhattan (i.e., close to the World Trade Center) during the terrorist attack had more detailed memories three years later (Sharot et al., 2007). This finding has been interpreted in terms of elevated noradrenergic arousal shortly after the terrorist attack (McGaugh, 2013). Remarkably, the "downtown participants" showed selective amygdala activation during the recall of events from 9/11 (Sharot et al., 2007), supporting the proposed model in Figure 13. As another example, crime victims often vividly remember the weapon – although they tend to forget other details, like the perpetrator's face (Steblay, 1992). This example highlights that noradrenergic arousal strengthens some (but not all) aspects of memory and can even lead to biases in long-term memories (Mather & Sutherland, 2011). Together, these observations illustrate how noradrenergic

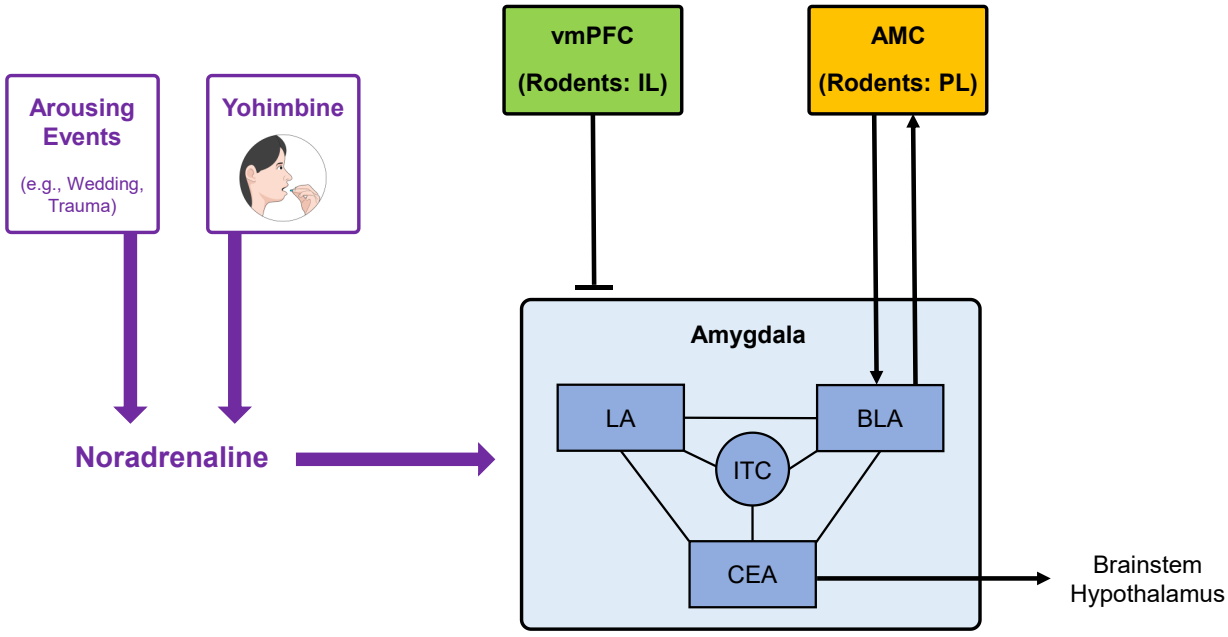


Figure 13. Highly arousing events (e.g., one's wedding, traumatic experiences) are supposed to activate cortico-subcortical circuits (e.g., amygdala, vmPFC/IL, AMC/PL) that are also involved in threat processing (LaLumiere et al., 2017; Mather et al., 2016; McGaugh, 2015). These effects are mediated by noradrenergic release. The brain circuits that are displayed in the right half are explained in more detail in Figure 4 (see also: Levy & Schiller, 2021; Pitman et al., 2012). Yohimbine can be used as an experimental tool to mimic the effects of noradrenergic arousal.

hyperactivity after fear acquisition may lead to pathological fear, which is often irrationally high, exaggerated, and also generalizes to other stimuli (Bowers & Ressler, 2015). Using the example of PTSD, environmental cues can be conceptualized as CSs, while the traumatic event would be the US in the conditioning terminology. As displayed in Figure 13, *yohimbine* can *experimentally mimic* the effects of emotional arousal in healthy participants. Thus, the noradrenergic substance yohimbine could be a promising experimental tool to investigate noradrenergic effects in the etiology of pathological fear (e.g., PTSD or specific phobias).

Besides its effects on fear consolidation, yohimbine has also been studied as a drug to boost extinction learning (Bowers & Ressler, 2015; Holmes & Quirk, 2010). Pathological fear is not only characterized by heightened consolidation, but also by impaired extinction learning (Duits et al., 2015; Miedl et al., 2020; Visser, 2020). Pharmacological facilitation of extinction mechanisms could be an innovative approach to improve exposure therapy for those patients who do not benefit sufficiently from standard procedures (Lebois et al., 2019; Parsons & Ressler, 2013). Yohimbine seems to enhance extinction learning in animals (Cain, 2004; Fitzgerald et al., 2014), even in rodents with extinction deficits (Hefner et al., 2008). Research in humans, however, has revealed mixed findings. Yohimbine improved the effects of exposure therapy in claustrophobia (Powers et al., 2009), social anxiety disorder (Smits et al., 2014), and PTSD (Tuerk et al., 2018), but was not beneficial to facilitate exposure therapy in patients with acrophobia (Meyerbroeker et al., 2018) and fear of flying (Meyerbroeker et al., 2012; Meyerbroeker et al., 2018).

To better understand these contradictory findings, it is necessary to elucidate and to disentangle yohimbine effects in well-controlled fear conditioning and extinction paradigms. In manuscript 4 (Sperl et al., submitted), we aimed to assess how yohimbine *differentially* modulates fear consolidation and extinction learning. Those brain regions that seem to be critical for yohimbine effects are part of circuits that are of particular relevance for threat processing (see Figure 13). Given this neurobiological overlap, we used EEG to capture yohimbine effects on conditioned threat responses.

Yohimbine does not only act at noradrenergic receptors; it also has antagonist properties at dopaminergic D2 receptors (Holmes & Quirk, 2010; Millan et al., 2000; Scatton et al., 1980). Potential dopaminergic influences have been ignored in most previous fear conditioning studies that investigated yohimbine effects. Yohimbine could block D2 autoreceptors, an action that would be associated with elevated cortical levels of dopamine (Gobert et al., 1997, 1998; Holmes & Quirk, 2010). This alternative explanation (i.e., dopaminergic instead of noradrenergic effects) would be quite plausible: As explained above, dopamine has also been shown to modulate emotional memories (Papalini et al., 2020; see schematic illustration in Figure 11). Furthermore,

dopamine has recently been proposed as a key mediator for the formation of long-term extinction memories (Gerlicher et al., 2018; Kalisch et al., 2019). Indeed, the dopaminergic substance sulpiride seems to facilitate extinction learning in mice (Ponnusamy et al., 2005; but: Shi et al., 2017; Vita et al., 2021). In manuscript 4 of this thesis (Sperl et al., submitted), we also aimed to disentangle noradrenergic and dopaminergic effects of yohimbine. To address this issue, we not only compared the yohimbine group with a placebo group; we also evaluated a third group that received sulpiride, which also allowed us to draw conclusions about dopaminergic effects. Sulpiride does not significantly block other (e.g., noradrenaline) receptor types (Caley & Weber, 1995; O'Connor & Brown, 1982). If yohimbine effects are genuinely mediated by noradrenergic mechanisms, effects should thus be specific for the yohimbine group and should be absent in the sulpiride group. It is essential to consider that sulpiride effects on pre- or postsynaptic receptors (Crockett & Fehr, 2014; Holmes & Quirk, 2010) depend on the chosen dose (Dubrovina & Zinov'eva, 2010; Ford, 2014; Mueller et al., 2010; Rankin et al., 2010; Stockhorst & Antov, 2015; Yim et al., 2009). High doses (i.e., > 400 mg) primarily act antagonistically on postsynaptic D2 receptors (Boschen et al., 2015; Eisenegger et al., 2014), which reduces dopaminergic actions (Lai et al., 2013). Low doses (i.e., 100–200 mg), however, block primarily presynaptic autoreceptors, leading to enhanced dopaminergic transmission (Kuroki et al., 1999; Tagliamonte et al., 1975). Based on our assumptions described above, we were interested in a net *stimulatory* effect on dopamine. To increase dopaminergic transmission experimentally (Kuroki et al., 1999; Mereu et al., 1983), we applied a relatively low dose of 200 mg (Chavanon et al., 2013; Mueller et al., 2011; Ohmann et al., 2020).

In addition to pharmacological manipulations, evaluating genetic polymorphisms is another approach to test hypotheses regarding associations between catecholamines and threat processing (Bomyea et al., 2012; VanElzakker et al., 2014). In manuscript 5 of the present thesis (Panitz, Sperl et al., 2018), we explored associations between the *COMT* Val158Met polymorphism and fear conditioning/extinction (see red arrows in Figure 11). This genetic polymorphism is associated with a substantial (three- to fourfold) variation in COMT enzyme activity (Lachman et al., 1996). COMT is an enzyme that is involved in the breakdown of catecholamines (Eisenhofer et al., 2004; Matsumoto et al., 2003). COMT facilitates the degradation of dopamine and, to a lesser extent, of noradrenaline (Barnett et al., 2011). The *COMT* gene is located on chromosome 22q11.2 (Bassett & Chow, 2008), as shown in Figure 14A. The Val158Met polymorphism is associated with an amino acid substitution at codon 158 from valine (Val) to methionine (Met), leading to significantly reduced COMT enzyme activity, less efficient catabolism, and higher catecholamine levels (Chen et al., 2004; Lachman et al., 1996; Lotta et al., 1995). The *COMT*

Val158Met polymorphism has been primarily linked to prefrontal dopamine activity (Bilder et al., 2004), which is further illustrated in Figure 14B. Met/Met (compared with Val/Val) homozygotes are characterized by a reduction in COMT activity, resulting in higher prefrontal dopamine activity. The alleles are codominant, and intermediate activity levels can be observed in Val/Met heterozygotes (Weinshilboum et al., 1999).

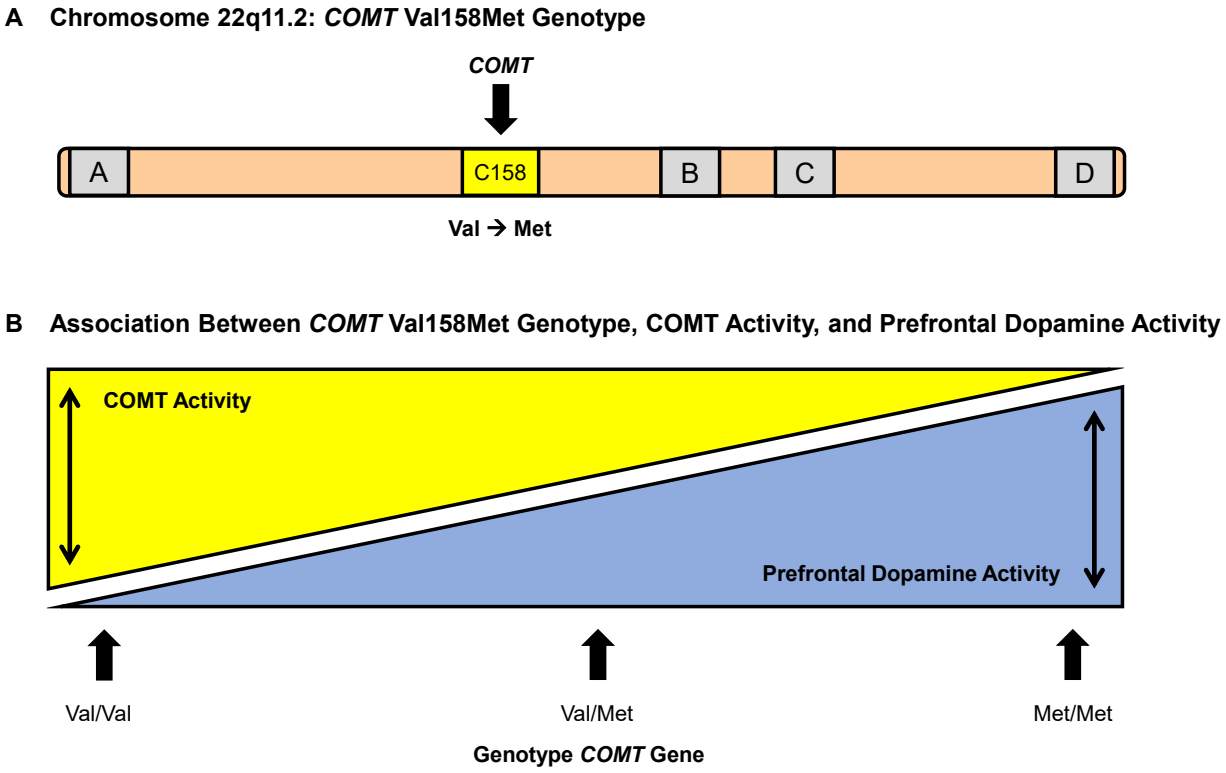


Figure 14. (A) The catechol-*O*-methyltransferase (*COMT*) gene is located on chromosome 22q11.2. A polymorphism at codon 158 is associated with a valine (Val) to methionine (Met) amino acid substitution (Jonas et al., 2014; Schacht, 2016). (B) As illustrated by Billino et al. (2016, 2017), genotypes of the *COMT* Val158Met polymorphism modulate COMT enzyme activity. COMT activity is assumed to be the major breakdown mechanism for dopamine in the prefrontal cortex. Compared with Val/Val homozygotes, Met/Met homozygotes show reduced COMT enzyme activity, resulting in elevated prefrontal dopamine activity. Val/Met heterozygotes are characterized by intermediate COMT and dopamine activity levels.

In manuscript 5 (Panitz, Sperl et al., 2018) of this thesis, we utilized this relationship between the *COMT* Val158Met polymorphism and catecholaminergic activity to explore catecholaminergic mechanisms involved in fear conditioning. Individual differences related to the *COMT* Val158Met polymorphism might elucidate the potential role of catecholamines to explain why certain individuals develop fear-related disorders after a traumatic event (i.e., a CS-US coupling) while

others are resilient (see Figure 11). In rodents, Met/Met compared with Val/Val homozygotes show increased fear recall and reduced extinction retention, suggesting that the Met allele might play a role in the etiology of fear-related disorders (Risbrough et al., 2014). This finding would be consistent with the observations pointing toward a higher risk for PTSD in Met carriers (Danzi & La Greca, 2018; Kolassa et al., 2010; Valente et al., 2011). Similarly, other authors have found links between the Met allele and obsessive-compulsive disorder (Gatt et al., 2015). Furthermore, Met/Met patients with panic disorder may benefit less from exposure therapy (Lonsdorf et al., 2010).

Converging with these findings, Met/Met carriers seem to show increased reactivity to unpleasant stimuli in the amygdala and prefrontal cortex (Smolka et al., 2005) – brain regions that are critical for threat processing (see Figure 4 in chapter 1.2). Lonsdorf et al. (2009) investigated the role of *COMT* Val158Met polymorphism on fear extinction in healthy humans and demonstrated that Met/Met homozygotes are characterized by impaired fear extinction. Similarly, Norrholm et al. (2013) found impaired extinction learning in Met/Met subjects with PTSD. However, associations between fear conditioning/extinction and the *COMT* Val158Met polymorphism seem to be more complex (Agren et al., 2012), and additional studies have revealed mixed and contradictory findings. Specifically, reduced aversive discrimination has been reported for Met carriers (Gruss et al., 2016). Other researchers were even unable to find robust associations between fear conditioning/extinction and the *COMT* Val158Met polymorphism (Klucken, Kruse et al., 2016; Raczka et al., 2011). Two hypotheses have been suggested to explain these inconsistent findings. First, while the authors of several human studies have investigated *COMT* Val158Met effects on within-session extinction or fear retention (see above), most of them did not assess *long-term* fear extinction with a delayed extinction retention test (Wilker et al., 2014). This issue is of particular relevance given that the rodent study described above found *COMT* Val158Met effects on extinction retention as assessed 24 hours after extinction training (Risbrough et al., 2014). Second, elevated threat responses during extinction training do not necessarily reflect impaired extinction learning, but could also be related to better consolidation of fear memory traces in Met/Met participants (LaLumiere et al., 2005; Lonsdorf & Kalisch, 2011). We considered both suggestions in manuscript 5 (Panitz, Sperl et al., 2018) of the present thesis. On the one hand, we assessed *COMT* Val158Met effects on *long-term* fear extinction, as assessed 24 hours after extinction training. On the other hand, we used an experimental design (Mueller et al., 2014) that allows differentiating between mechanisms related to *fear consolidation* (“fear recall”) and *extinction learning* (“extinction recall”).

1.7 The Present Thesis – Research Questions

Ressler and colleagues (Bowers & Ressler, 2015; Maddox et al., 2019; Parsons & Ressler, 2013; Ross et al., 2017) have introduced an integrative model (see Figure 15) that describes the development and maintenance of fear-related disorders. This model aims to synthesize key etiological mechanisms. Thereby, this model integrates findings from previous research and opens new avenues for translationally informed treatment strategies. The starting point of this model is the assumption that specific individuals are *predisposed* (based on their genetic background, as discussed in chapter 1.6) to develop pathological fear. It is critical to emphasize that genetic predispositions do not necessarily lead to the development of a disorder. However, the interaction of a preexisting sensitivity and *(pathological) learning* (e.g., the experience of a traumatic event) can be critical etiological factors.

In this context, fear conditioning mechanisms are particularly important, as discussed in the previous chapters. Regarding the example of a dog bite (US), a dog or other related stimuli (e.g., visual and auditory cues in the environment) can become CSs and thus elicit a conditioned fear response. However, the simple experience of a CS-US pairing is often not sufficient to develop fear-related disorders. Hyperconsolidation of aversive associations in the aftermath of aversive or traumatic experiences (in the range of hours to days) seem to be crucial, leading to pathological fear expression that is often characterized by nightmares, flashbacks, exaggerated startle responses, avoidance of CS-related situations (which trigger excessive threat responses), and chronic hyperarousal. According to the model of Ressler and colleagues (Bowers & Ressler, 2015; Maddox et al., 2019; Parsons & Ressler, 2013; Ross et al., 2017), pathological fear includes generalization (i.e., recruitment of non-associated cues, fear evoked by stimuli that are similar to the CSs) and sensitization (i.e., increased fear with repeated exposure to the CSs) processes. Conversely, successful recovery from or resilience against pathological fear are thought to be mediated by extinction processes (i.e., diminished response to CSs over time) and discrimination learning (i.e., limiting of fear to specific threat cues, in contrast to generalization). Exposure therapy consists of repeated confrontations with the feared stimulus, together with the experience that the feared outcome does not occur. This is a highly efficient way to achieve transitions from pathological fear to extinguished fear, including the ability to discriminate between non-threatening cues and real danger.

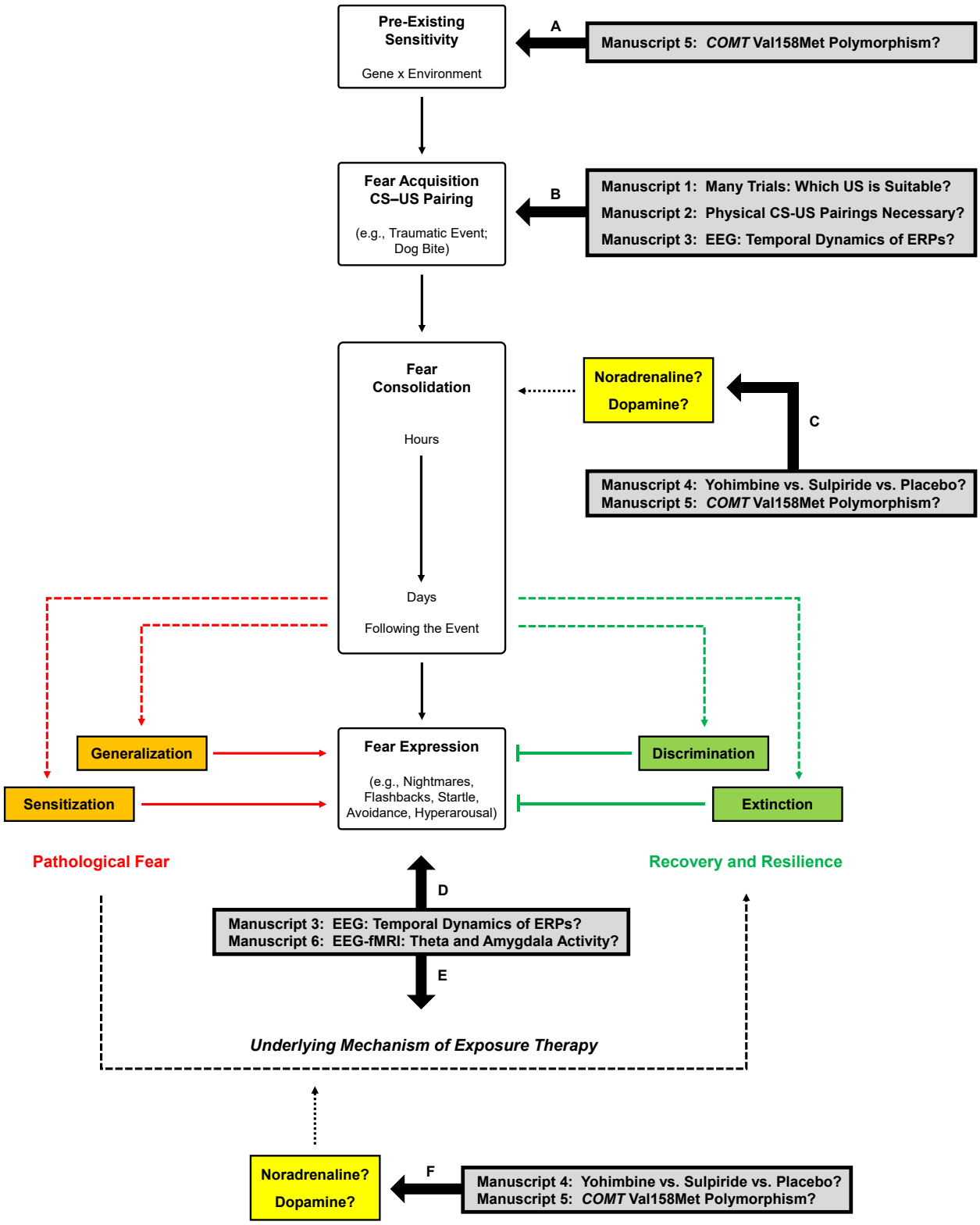


Figure 15. Ressler and colleagues (Bowers & Ressler, 2015; Maddox et al., 2019; Parsons & Ressler, 2013; Ross et al., 2017) introduced a model to explain the development of fear-related disorders from the perspective of fear conditioning processes. This model provides a framework to integrate the experimental studies that are part of this thesis (arrows A–F).

Importantly, the model by Ressler and colleagues (Bowers & Ressler, 2015; Maddox et al., 2019; Parsons & Ressler, 2013; Ross et al., 2017) provides a framework to integrate the six manuscripts that are part of this thesis, as illustrated in Figure 15. All studies used fear conditioning paradigms. This thesis closed methodological gaps, explored neurobiological and catecholaminergic mechanisms, and aimed to move forward with translational fear conditioning research.

According to the model by Ressler and colleagues (Bowers & Ressler, 2015; Maddox et al., 2019; Parsons & Ressler, 2013; Ross et al., 2017), the confrontation with an aversive stimulus is a key event in the etiology of pathological fear. This stimulus, which naturally triggers a threat response, can be conceptualized as a US. The goal of **manuscript 1** was to identify an appropriate US type for fear conditioning paradigms with many trials, as it is typically the case in EEG research (see ***arrow B*** in Figure 15). This study lays the groundwork for the subsequent studies of this thesis, as all other studies (except for manuscript 2) used EEG methods. The findings of manuscript 1 have been published in *Psychophysiology* (**Sperl**, Panitz, Hermann, & Mueller, 2016).

The model by Ressler and colleagues (Bowers & Ressler, 2015; Maddox et al., 2019; Parsons & Ressler, 2013; Ross et al., 2017) states that CS-US pairings are necessary for the development of pathological fear. In Figure 15, there is no arrow bypassing this second box. At first glance, this is in marked contrast to the experiences of many clinicians and patients who often cannot report such stimulus pairings (e.g., a dog bite in patients with dog phobia). Building on this apparent divergence, the aim of **manuscript 2** was to answer the questions of whether fear conditioning is also possible with an imagined US, without any physical stimulation (see ***arrow B*** in Figure 15). Related to clinical models, this would suggest that the repeated *imagery* of a dog bite could be sufficient for certain predisposed individuals. Manuscript 2 includes two experimental fear conditioning studies with USs that are only imagined, in the total absence of any physically aversive stimulation. The second study described in manuscript 2 is an extended replication of the first study in this manuscript. Manuscript 2 has been published in *Psychological Science* (Mueller, **Sperl**, & Panitz, 2019).

Furthermore, the model by Ressler and colleagues (Bowers & Ressler, 2015; Maddox et al., 2019; Parsons & Ressler, 2013; Ross et al., 2017) implies that fear responses increase through CS-US pairings. Figure 3 in chapter 1.1 suggests a linear increase in conditioned responses during fear acquisition – although in most studies researchers have not formally tested this linear growth. In typical EEG studies, in addition to waiving this formal test, the *increase* in conditioned responses during fear acquisition training is often completely ignored. To achieve an acceptable signal-to-noise ratio, a massive number of trials is usually averaged (Huffmeijer et al., 2014; Miskovic &

Keil, 2012; Steinberg et al., 2013), and these trials are typically weighted equally, completely neglecting the assumed linear increase, as displayed in Figure 3. In **manuscript 3** of the present thesis, we developed a new sequential-set fear conditioning design that allows to elucidate temporal dynamics and the learning curve of ERP components. Specifically, we illustrated how differential ERP responses increase and decrease during fear acquisition (see ***arrows B and D*** in Figure 15) and extinction (see ***arrow E*** in Figure 15), respectively. These results have been published in *NeuroImage* (**Sperl**, Wroblewski, Mueller, Straube, & Mueller, 2021).

In the etiology of fear-related disorders, CS-US pairings play an important role, albeit merely imagined stimuli can serve as USs (see manuscript 2). However, CS-US pairings are not *sufficient* for the development of fear-related disorders. The model by Ressler and colleagues (Bowers & Ressler, 2015; Maddox et al., 2019; Parsons & Ressler, 2013; Ross et al., 2017) implies that hyperconsolidation of these CS-US associations leads to pathological fear. Noradrenergic hyperarousal is thought to facilitate these consolidation processes (LaLumiere et al., 2017; McGaugh, 2015). Indeed, physiological arousal levels after traumatic experiences are often elevated, which may be of particular relevance for later psychopathology (Kapfhammer, 2013; McGaugh, 2013). Noradrenergic arousal does not only play a pivotal role in the etiology of exaggerated fear. In fact, elevated noradrenergic release during or after exposure therapy has been suggested to facilitate the consolidation of extinction memories and could thus facilitate the efficacy of psychotherapeutic interventions (Bowers & Ressler, 2015). As discussed in chapter 1.6, previous studies in clinical samples have revealed mixed findings regarding these hypotheses. There is a need to explore underlying basic mechanisms in standardized fear conditioning paradigms. This approach would allow researchers to isolate relevant mechanisms of action, which could be further utilized to improve clinical interventions. In **manuscript 4** of the present thesis, participants underwent fear conditioning and extinction and received the noradrenergic substance yohimbine (versus placebo). We used an established design (Mueller et al., 2014) to disentangle noradrenergic effects on fear consolidation and extinction learning (see ***arrows C and F*** in Figure 15). Central (electroencephalographic data, ERPs) and peripheral physiological measures were used to capture yohimbine effects. A third group received the dopaminergic substance sulpiride, which allows testing whether effects are specific for noradrenaline. Similarly to noradrenaline, dopamine has also been discussed to have significant actions on fear and extinction learning (Abraham et al., 2014; Kalisch et al., 2019; Luo et al., 2018). Manuscript 4 has been submitted to *Neuropsychopharmacology* (**Sperl**, Panitz, Skoluda, Nater, Pizzagalli, Hermann, & Mueller, submitted).

In addition to the experimental approach of manuscript 4 (pharmacological intervention: yohimbine versus placebo versus sulpiride), **manuscript 5** followed a quasi-experimental approach. In this study, subjects with different *COMT* genotypes were compared (see ***arrow A*** in Figure 15). Although this quasi-experimental design does not allow us to draw causal conclusions, we were able to further elucidate catecholaminergic mechanisms in fear conditioning and extinction (see ***arrows C and F*** in Figure 15). As outlined in chapter 1.6, the Val158Met polymorphism of the *COMT* gene is related to COMT-dependent degradation of dopamine and noradrenaline (Lachman et al., 1996; Lotta et al., 1995). In manuscript 5, we investigated whether participants with different *COMT* genotypes (associated with high versus low catecholaminergic activity) show altered conditioned responses during fear recall and extinction recall. We used the same experimental paradigm as in manuscript 4. In addition to the pharmacological manipulation study (manuscript 4), the investigation of genetic variations allowed us to further elaborate catecholaminergic (dopamine, noradrenaline) models of fear processing. In light of the etiological model introduced by Ressler and colleagues (Bowers & Ressler, 2015; Maddox et al., 2019; Parsons & Ressler, 2013; Ross et al., 2017), catecholaminergic modulations could be crucial for fear consolidation and extinction learning, as already explained above (see Figure 15). The findings of manuscript 5 have been published in *Neurobiology of Learning and Memory* (Panitz, **Sperl**, Hennig, Klucken, Hermann, & Mueller, 2018).

Manuscripts 4 and 5 aimed to elucidate catecholaminergic mechanisms in fear processing. As discussed in chapter 1.6, catecholamines are assumed to modulate activity in brain areas that are critical for fear-related processes, especially the amygdala and prefrontal areas (Figure 13). The "traditional" neurobiological model of threat processing (see chapter 1.2) states that bidirectional connectivity between the AMC and the amygdala (Figure 4) is important for maintaining *sustained* threat responses (Levy & Schiller, 2021; Pitman et al., 2012), presumably mediated through theta synchrony (Gilmartin et al., 2014). Due to methodological restrictions, the covariance of prefrontal theta oscillations and amygdala activity has not yet been studied in humans. In manuscripts 3, 4, and 5, we assessed ERP signatures of fear conditioning and extinction. However, as outlined in chapter 1.4, EEG data can also be analyzed in the *frequency* domain to study *oscillatory* brain activity. **In manuscript 6**, we used simultaneous EEG-fMRI recordings to reveal the expected interplay of amygdala activation and frontomedial theta oscillations during fear recall (see ***arrow D*** in Figure 15) and extinction recall (see ***arrow E*** in Figure 15). Manuscript 6 has been published in *Cerebral Cortex* (**Sperl**, Panitz, Rosso, Dillon, Kumar, Hermann, Whitton, Hermann, Pizzagalli, & Mueller, 2019).

2 Summaries of Empirical Studies

In the following chapters, the key findings of the empirical studies that are part of this thesis are summarized. The entire *Research Articles* are included in chapter 4 and *Supplementary Materials* are provided in chapter 5. Code-Books for *Open Data* and *Open Materials* (for manuscripts 2, 3, and 4) are available online at the *Zenodo* repository.

2.1 Manuscript 1:
A Pragmatic Comparison of Noise Burst and Electric Shock Unconditioned Stimuli for Fear Conditioning Research With Many Trials

Sperl, M. F. J., Panitz, C., Hermann, C., & Mueller, E. M. (2016). A pragmatic comparison of noise burst and electric shock US for fear conditioning research with many trials. *Psychophysiology*, *53*, 1352–1365. https://doi.org/10.1111/psyp.12677

The study described in manuscript 1 aimed to identify a US type that is well suited for fear conditioning paradigms with high numbers of trials. Importantly, this study lays the foundation for the other publications that are part of this thesis. In most studies of this thesis, we used EEG methods to investigate neurophysiological correlates of fear conditioning. As explained in chapter 1.4, EEG is a promising method to elucidate knowledge about brain *mechanisms* and the *temporal* dynamics of neural threat processing. In contrast to other neuroscientific techniques like fMRI, the EEG method requires a massive number of trials to achieve an acceptable signal-to-noise ratio (Huffmeijer et al., 2014; Miskovic & Keil, 2012; Steinberg et al., 2013). Averaging across a high number of trials, however, can be problematic, as unconditioned responses to the US may habituate over time. If the US is no longer perceived as unpleasant during late acquisition trials, extinction processes may already emerge during these trials. This can dramatically reduce the power of fear conditioning paradigms and even lead to invalid conclusions. Thus, the following question arises: Which US type is best suited for fear conditioning paradigms with EEG, which are typically characterized by many trials? According to several meta-analyses and reviews (Duits et al., 2015; Fullana et al., 2016; Hofmann et al., 2010; Lissek et al., 2005; Mechias et al., 2010; Shechner et al., 2014), electric shocks and white noise bursts are among the most frequently used US types in human fear conditioning studies. Several studies have compared different US types in animals and humans using fear conditioning paradigms with *fewer* acquisition trials (Busch & Evans, 1977;

Glenn et al., 2012; McEchron et al., 1992; Murray & Carruthers, 1974; Neumann & Waters, 2006). However, it remains unclear which US is best suited for fear conditioning paradigms with a *high* number of trials, which is particularly the case in EEG research. In fact, EEG researchers are often faced with the rather pragmatic question of which stimuli might be most suitable to produce fear conditioning effects. The evocation of robust conditioned responses is a prerequisite for detecting reliable and valid EEG correlates. In manuscript 1, we applied a fear conditioning paradigm that has been specifically developed for EEG research (Mueller et al., 2014). This paradigm (in a slightly modified form) was further used in manuscripts 4, 5, and 6 of this thesis. In fact, the first study was explicitly designed to identify an appropriate US type for the other studies of this thesis.

To address this question of the most appropriate US type, we applied a between-subjects design. Specifically, $N = 32$ participants were randomly assigned to two experimental groups. The first group underwent fear conditioning with an electric shock US, while a white noise burst US was used for the second group. Both US types were presented in the way they are typically applied in current research. The electric shock US was a 500-ms multipulse percutaneous stimulation that was delivered from a constant current stimulator via two steel disk electrodes fixed to the inside of the left forearm. A work-up procedure was performed to set shock intensities to a level that was rated as "highly annoying but not painful." This procedure (i.e., the adjustment of gradually increasing shocks to an individual threshold) is very common in fear conditioning research (e.g., Coppens et al., 2009; Hermann et al., 2016; Martínez et al., 2014; Merz et al., 2016; Milad, Quirk et al., 2007; Weike et al., 2007). By contrast, fear conditioning studies that use a noise burst US typically do not use such a work-up procedure. Instead, the noise burst is usually presented in a predefined intensity (e.g., Critchley et al., 2002; Dolan et al., 2006; Moses et al., 2007; Mueller et al., 2014; Mueller & Pizzagalli, 2016; Peri et al., 2000). Thus, the second group received a loud 95 dB white noise burst US, with a duration of 1,000 ms. The noise burst was presented over headphones. The sound pressure level was reduced to 92 dB when subjects perceived the 95 dB burst as too loud.

Participants underwent a two-day fear conditioning and extinction paradigm (Mueller et al., 2014), which is illustrated in Figure 16. Habituation, acquisition, and extinction phases took place on day 1. The recall of conditioned and extinguished fear was assessed approximately 24 hours later. Pictures of male faces with a neutral expression were used as CSs, as these stimuli are particularly prepared for fear conditioning (Kret & Gelder, 2012; Mazurski et al., 1996; Öhman & Dimberg, 1978). This experimental paradigm allowed us to disentangle mechanisms involved in fear recall from mechanisms underlying extinction recall. This differentiation is of particular

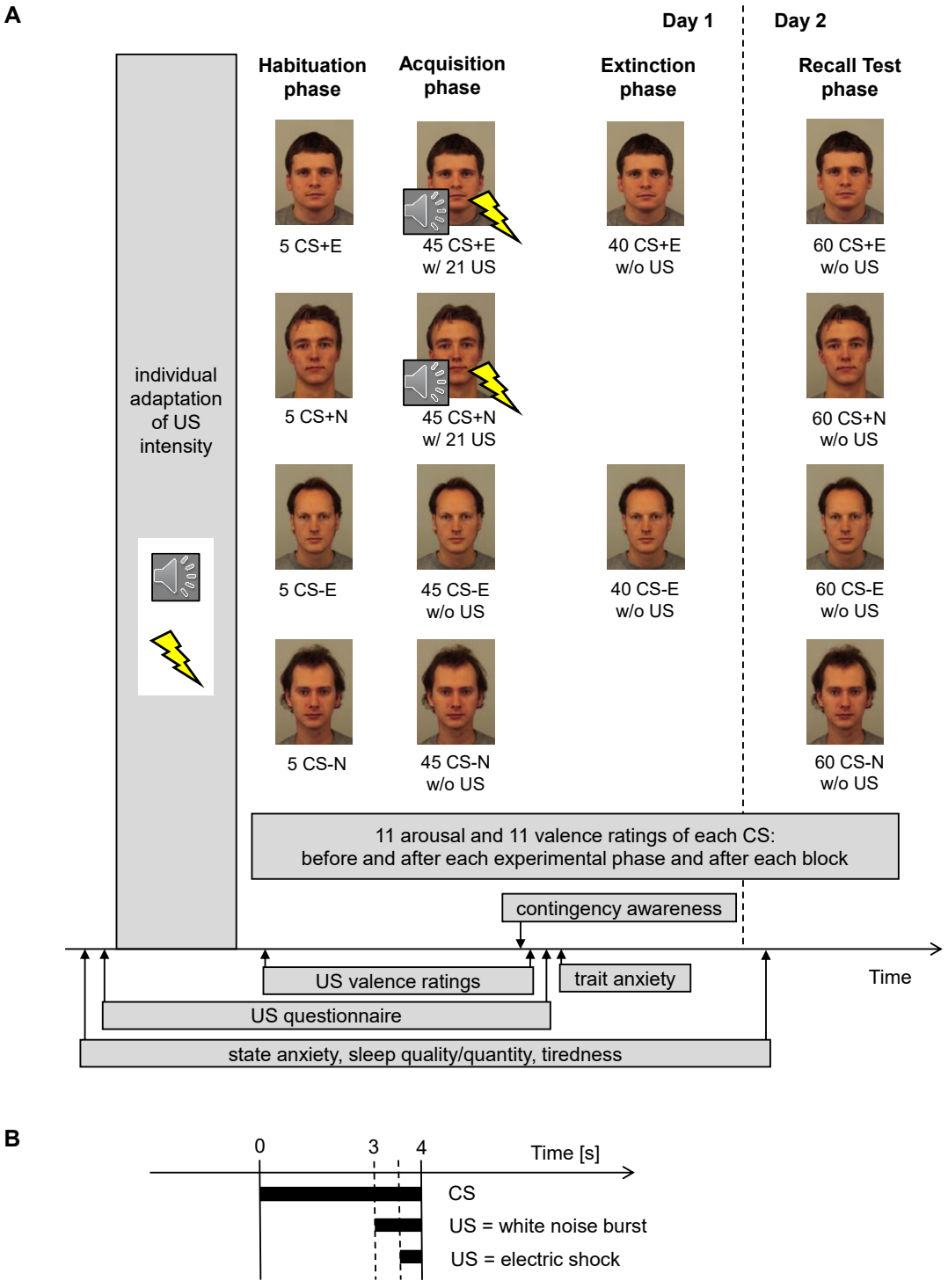


Figure 16. Experimental design used in the study described in manuscript 1. (A) The number and types of stimuli presented during the experimental phases. Face stimuli are taken from Lundqvist (1998), IDs: AM10NES, AM13NES, AM31NES, BM08NES. Extinguished stimuli (CS+/-E) were presented during all phases, while nonextinguished stimuli (CS+/-N) were not presented during the extinction phase. For results regarding the US questionnaire, sleep quality/quantity, tiredness, and trait/state anxiety, see the original manuscript in chapter 4. (B) Trial structure. Stimulus duration differed between both CS+ types and was similar to those durations that are typically used in fear conditioning studies with the respective US type. In the fear conditioning literature, the duration of electric shock USs is usually shorter than white noise burst durations. Figure republished from Sperl et al. (2016).

relevance for manuscripts 4, 5, and 6. We used two CS+ (CS+E, CS+N) that co-terminated with the aversive US during acquisition training at a partial reinforcement rate of 47%. Two CS- (CS-E, CS-N) were never paired with the US. During extinction training, only one of the two CS+ (i.e., the "extinguished" CS+, the CS+E) and one of the CS- (i.e., the CS-E) were shown, and responses to those stimuli aimed to be extinguished. Conversely, the other CS+ (i.e., the "nonextinguished" CS+, the CS+N) and the other CS- (i.e., the CS-N) were not shown during extinction training, with the intention to leave learned responses to those stimuli fully intact. Twenty-four hours later, all stimuli were shown again without US presentation. The comparison of extinguished (CS+E versus CS-E) and nonextinguished (CS+N versus CS-N) stimuli on the second day allowed us to identify effects specific to extinction versus fear recall. The differentiation between fear recall and extinction recall is less relevant for manuscript 1, but essential for manuscripts 4, 5, and 6.

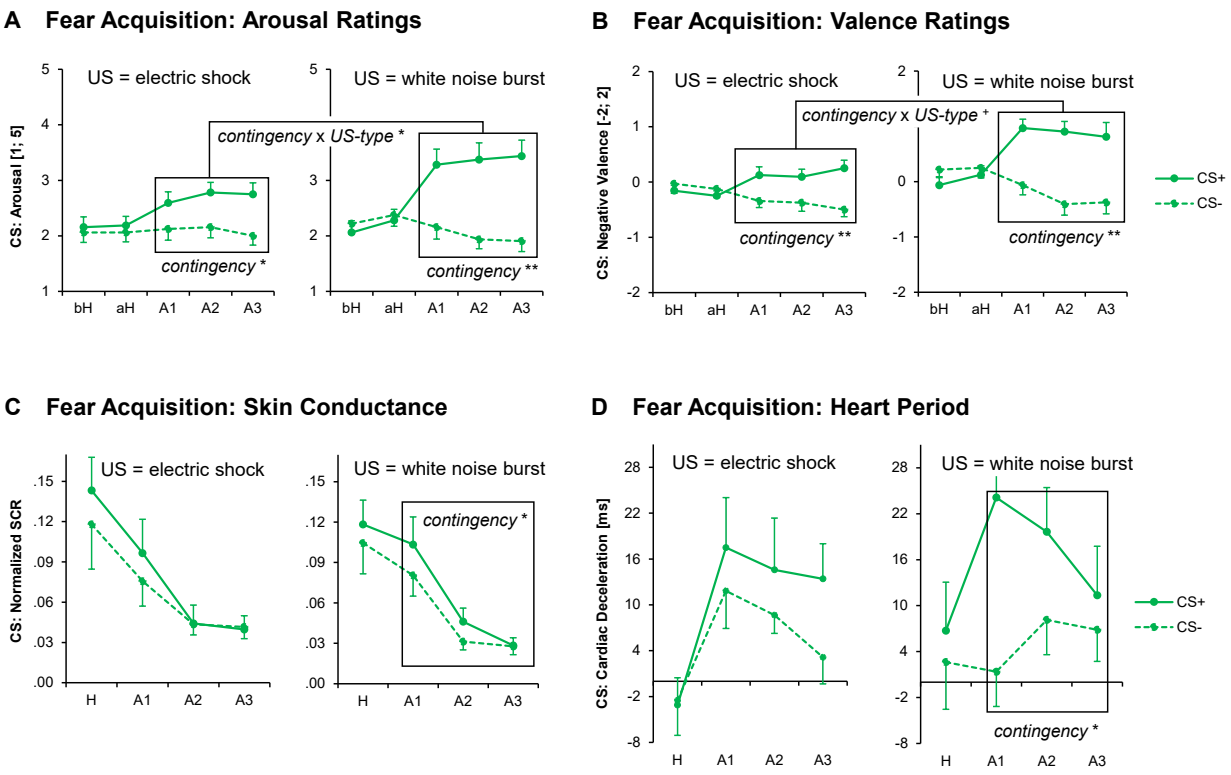


Figure 17. Fear conditioning with a white noise burst US (compared with an electric shock US) led to superior fear conditioning, as measured with affective CS ratings and peripheral physiology. (A) Subjective CS arousal ratings from 1 ("not arousing"), to 5 ("very arousing"); (B) subjective CS valence ratings from -2 ("very pleasant") to 2 ("very unpleasant"); (C) normalized CS-evoked skin conductance response (SCR) amplitudes; and (D) CS-evoked heart period changes during habituation and acquisition phases (day 1). bH/aH = before/after habituation phase (arousal/valence); H = during habituation phase (SCR, heart period); A1, A2, A3 = after (arousal/valence), respectively, during (SCR, heart period) acquisition block 1, 2, 3; line charts indicate $M \pm SEM$. $^{+}p < .10$; * $p < .05$; ** $p < .01$. Figure republished from Sperl et al. (2016).

To quantify conditioned responses, participants were asked to rate perceived arousal from 1 ("not arousing") to 5 ("very arousing") and valence from -2 ("very pleasant") to 2 ("very unpleasant") for each CS at various times during the experiment. Furthermore, we assessed SCRs and heart period changes. All measures pointed toward superior fear acquisition when the white noise burst (compared with the electric shock) was used as US. As displayed in Figure 17, differential (CS+ versus CS-) responses measured with arousal and valence ratings were larger in the white noise group than in the electric shock group. Regarding SCRs and fear-conditioned bradycardia (heart rate deceleration), robust conditioned responses could only be measured in the white noise group; they were absent in the electric shock group. Moreover, conditioned responses in the white noise group were more resistant to extinction, and recall of conditioned fear was greater 24 hours later (for statistical details, see chapter 4.1), as indicated in Figure 18.

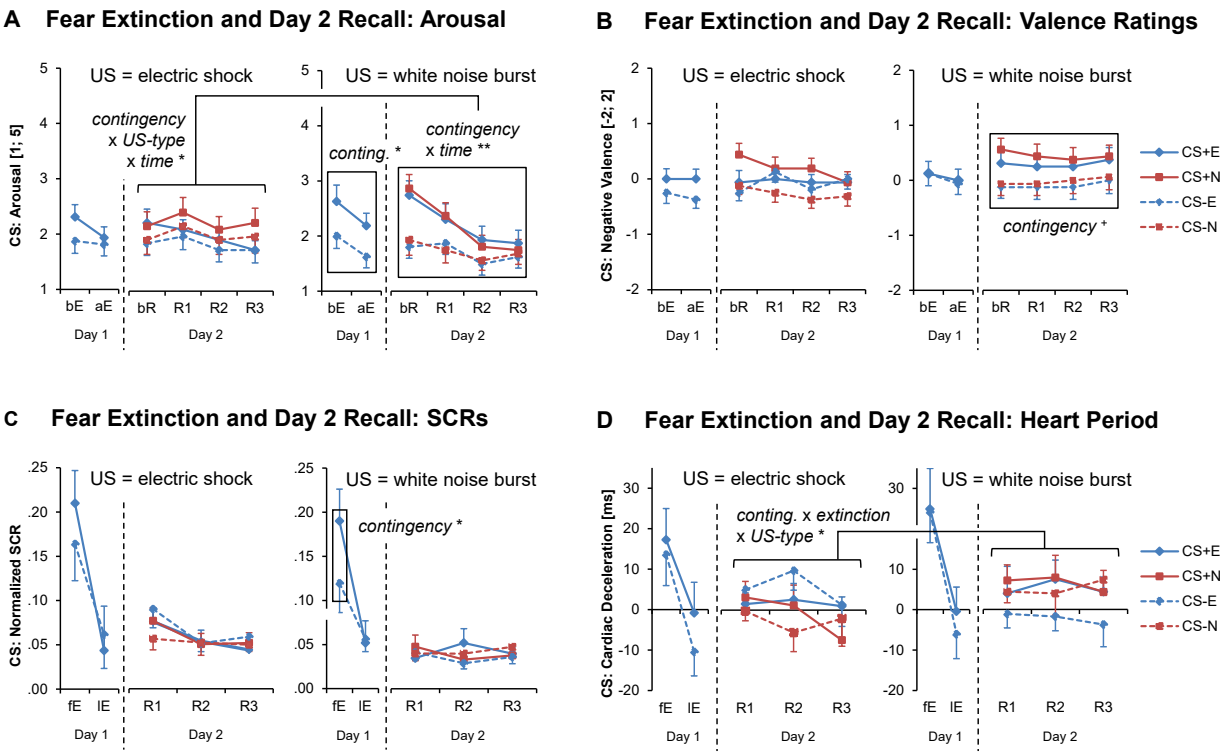


Figure 18. Fear conditioning with a white noise burst US (compared with an electric shock US) led to more extinction-resistant conditioned responses and elevated fear recall 24 hours later. (A) Subjective CS arousal ratings from 1 ("not arousing") to 5 ("very arousing"); (B) subjective CS valence ratings from -2 ("very pleasant") to 2 ("very unpleasant"); (C) normalized CS-evoked skin conductance response (SCR) amplitudes; and (D) CS-evoked heart period changes during extinction (day 1) and recall test (day 2) phases. CS+N and CS-N: nonextinguished stimuli; CS+E and CS-E: extinguished stimuli; bE/aE = before/after extinction phase; fE/lE = during the first/last ten extinction trials; R1, R2, R3 = after (arousal/valence), respectively, during (SCR, heart period) recall test block 1, 2, 3; line charts indicate $M \pm SEM$. $^{+}p < .10$; * $p < .05$; ** $p < .01$. Figure republished from Sperl et al. (2016).

The valence of the US was also rated before the habituation stage and after acquisition training from 0 (“not unpleasant at all”) to 10 (“extremely unpleasant”). Ratings of the US revealed that the valence of the white noise US remained unpleasant throughout fear acquisition, but declined for the electric shock. These results suggest stronger habituation of unconditioned responses in the electric shock group.

It is important to note that the goal of this study was *not* to clarify underlying mechanisms. These may be related to habituation processes (Çevik, 2014; Dycus & Powers, 2000; Jordan et al., 2015); different US durations (Gallistel & Gibbon, 2000; Lipp, 2006; Marter et al., 2014); different belongingness levels (Hamm et al., 1989); or different contributions of neural systems, as suggested by the “two-system” framework, which has been introduced at the beginning of this thesis (LeDoux, 2017; LeDoux & Daw, 2018; LeDoux & Pine, 2016). Instead, we aimed to compare two common US types in the way they are typically applied in current research. This includes different work-up procedures for both stimuli, which is also related to guidelines of institutional review boards.

Manuscript 1 laid the foundation for choosing appropriate US types in all other manuscripts of this thesis. Building on these findings, we used a white noise US in the EEG studies that are described in manuscripts 4 and 5. Manuscript 6 summarizes findings from a simultaneous EEG-fMRI study. Due to the enormous noise in the MRI environment (caused by the MRI method), acoustic stimuli are difficult to deliver in fMRI studies. Thus, we used an electric shock US in the study that is described in manuscript 6. However, based on our findings from manuscript 1, we chose a relatively high shock intensity to prevent habituation to the US. Instead of using a shock intensity that is “highly annoying but not painful” (manuscript 1), shock intensities were set to a level that is “difficult to bear, but acceptable” (manuscript 6). Importantly, we showed successful fear conditioning with this high-intensity shock US. The same shock US (“difficult to bear, but acceptable”) was used in the third manuscript of this thesis. In manuscript 3, we developed a new sequential-set fear conditioning paradigm for EEG research, which is specially designed to capture habituation-prone (e.g., amygdala-related; Armony & Dolan, 2001; Büchel et al., 1998, 1999; Yin et al., 2018) neural processes. For future studies, it is of particular interest to adapt this design for the MRI environment, which would allow better imaging of amygdalar activity (Geissberger et al., 2020; Janak & Tye, 2015; Patin & Hurlemann, 2011). To be able to use this sequential-set conditioning design in the MRI environment in the future, a (high-intensity) electric shock US (in contrast to a white noise burst US) was more practicable for manuscript 3.

2.2 Manuscript 2:
Aversive Imagery Causes De Novo Fear Conditioning

Mueller, E. M., **Sperl, M. F. J.**, & Panitz, C. (2019). Aversive imagery causes de novo fear conditioning. *Psychological Science*, *30*, 1001–1015.
https://doi.org/10.1177/0956797619842261

Open Data and **Open Materials** available online at *Zenodo*:
https://doi.org/10.5281/zenodo.2591593

In manuscript 1, our goal was to identify an aversive stimulus that is suitable to be used as a US in fear conditioning studies. A common implicit assumption in fear conditioning research is that the US needs to be an unpleasant *physical* stimulus (e.g., electric shock, dog bite, traumatic event). Regarding the external and ecological validity of the fear conditioning paradigm (as a model for pathological fear and anxiety disorders), this assumption can be highly problematic. Indeed, several patients with anxiety disorders *cannot* recall autobiographical experiences (i.e., confrontations with an aversive US) that are temporally linked to symptom onset (Harvey et al., 2005; Hofmann et al., 1995; Moscovitch et al., 2011; Murray & Foote, 1979; Rachman, 1977). This discrepancy raises an important question: Is the confrontation with a *physical* aversive US – as suggested by the etiological model of Ressler and colleagues (Bowers & Ressler, 2015; Maddox et al., 2019; Parsons & Ressler, 2013; Ross et al., 2017; see Figure 15 in chapter 1.7) – really a necessary prerequisite for fear conditioning?

Direct aversive conditioning experiences seem to be one, *but not the only* causal factor to explain the etiology of anxiety and fear-related disorders. In addition to direct CS-US pairings, fear acquisition can also take place without direct contact with the feared stimulus. Observational, vicarious, and instructional learning experiences have been discussed as relevant mechanisms (Haaker et al., 2017; Keum & Shin, 2019; Mineka et al., 1984; Olsson et al., 2007; Olsson & Phelps, 2007). Aversive *mental imageries* might be another etiological factor to develop anxiety disorders (Blackwell, 2021; Hendrikx et al., 2021; Krypotos et al., 2020; Mertens et al., 2020). Although others have studied imagined USs in fear conditioning (Dadds et al., 1997; Mertens et al., 2020), it remains unclear whether mental images of an unpleasant US cause *de novo* fear conditioning even without explicit CS-US contingency instructions (Arabian, 1982; Soeter & Kindt, 2012a) and in the total absence of any physical US presentation (Jones & Davey, 1990). Translating this research question to an everyday life example would mean: Can individuals develop dog phobia because of *aversive imagery* when being confronted with a dog (i.e., the

imagery of being bitten by this dog), although they have never been physically attacked by a dog? With manuscript 2, we closed this gap. In two consecutive studies, we asked the question of whether fear can be conditioned with aversive mental images as USs only.

To this end, participants were first trained to produce specific mental images (an aversive image, a neutral image, or no-image) when particular geometric figures were shown (see Figure 19A). For example, participants were instructed to produce an aversive imagery (i.e., the aversive US) when a red square was shown. When a yellow hexagon was presented, "no" image should be retrieved (no-US; "you do not have to imagine anything"). To control for the potential influence of images per se (regardless of their valence), participants were instructed to imagine a neutral situation when a blue ellipse was presented (neutral US). The assignment of imagery scripts to geometric figures was counterbalanced. Afterward, during fear acquisition (see Figure 19B), CSs were systematically paired with these imagery cues. The actual USs should be imagined upon the appearance of the geometric cues. As in manuscript 1, pictures of male faces with a neutral expression served as CSs. The "aversive CS+" was paired with the cue for aversive imagery (e.g., a red square), the "neutral CS+" was paired with the cue for neutral imagery (e.g., a blue ellipse), and the "CS-" was paired with the cue for no-imagery (e.g., a yellow hexagon). After fear acquisition, extinction learning was also assessed. To capture conditioned responses (time-locked to the CS faces) and unconditioned responses (time-locked to the imagery cues), we collected affective ratings, heart period changes, SCRs, and eyelid startle magnitudes.

Manuscript 2 includes two studies that differ mainly in the imagery scripts used. In the first study, participants were instructed to imagine stepping on a *thumbtack* (aversive US) whenever a particular geometric shape was presented:

> *Imagine the following situation: You walk barefoot through a room, and your right foot steps on a thumbtack. You can feel the thin needle sinking into your heel as you step on the pin with your entire weight. The pain is piercing and intense and spreads from your heel into your leg. Every [red square/blue ellipse/yellow hexagon] that appears on the screen evokes the feeling of the needle pushing into your heel and the piercing and intense pain going through your body. The stinging pain is extremely unpleasant and barely tolerable. Focus on the pain you are experiencing. You can feel how it spreads from your right heel and you are cramping. You do not want to experience the stinging pain again. With every [red square/blue ellipse/yellow hexagon], you feel the thumbtack pushing into your heel.*

To differentiate CS responses associated with aversive imagery from CS responses related to imagery per se, participants were instructed to imagine stepping on a *coin* (neutral US) when another geometric shape was shown:

> *Imagine the following situation: You walk barefoot through a room, and your right foot steps on a 1-cent coin. You can feel the round metal under your heel when you step on it. The coin feels*

cool but it is not unpleasant. Every [red square/blue ellipse/yellow hexagon] that appears on the screen evokes the feeling of the round, cool coin under your heel. The contact is not unpleasant and is easily tolerable. Focus on the contact; you are relaxed. With every [red square/blue ellipse/yellow hexagon] that appears on the screen, you feel the round, cool coin under your heel.

The script for the control cue was as follows (*no-imagery* instruction, no US):

Whenever this [red square/blue ellipse/yellow hexagon] appears on the screen, you do not have to imagine anything. Just sit in your chair, observe the [red square/blue ellipse/yellow hexagon], and think of nothing in particular.

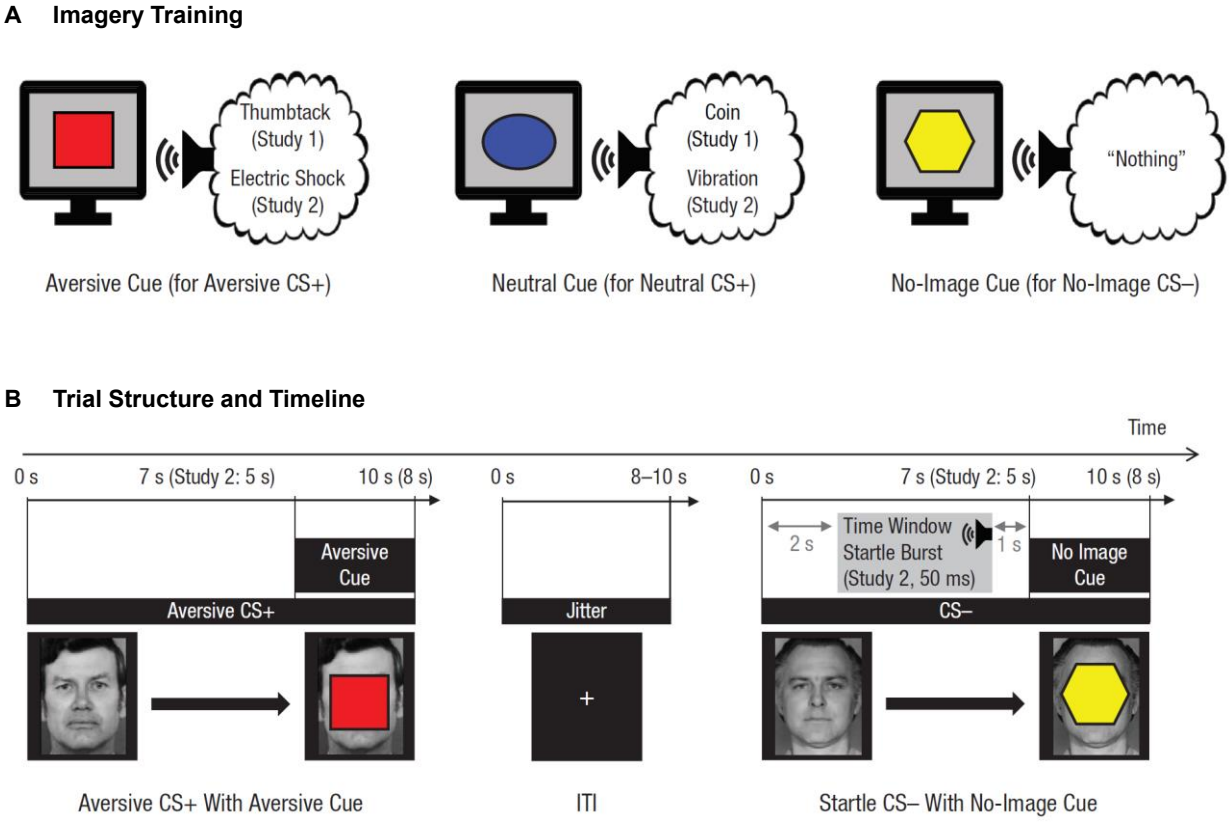


Figure 19. Schematic depiction of the experimental protocol used in both studies that are described in manuscript 2. (A) During imagery training, participants were informed of the association between cues (e.g., square, ellipse, hexagon) and imagery scenarios of aversive valence (e.g., stepping on a thumbtack) or neutral valence (e.g., stepping on a coin). In a third condition, participants were instructed not to imagine anything. (B) During the imagery-based differential-conditioning procedure, each of three neutral faces (aversive CS+, neutral CS+, and CS-) was paired with the corresponding geometric cue (aversive cue, neutral cue, no-image cue). All CSs were presented for 10 s (first study) or 8 s (second study). CS presentations co-terminated with the imagery cue centrally superimposed on the CS for the last 3 s in 80% of the trials. In the second study, acoustic startle probes were presented during 50% of CS presentations (potential window: 2–4 s after CS onset, i.e., prior to the onset of the imagery cue) and during six intertrial intervals (ITIs). The extinction phase was identical to the acquisition phase, except that the imagery cues were never shown. Figure republished from Mueller, Sperl, & Panitz (2019).

Subjective cue ratings and physiological responses to the cues indicated that the aversive cue (in contrast to the neutral cue and the no-image cue) was associated with highly unpleasant imagery. The aversive imagery was accompanied by heart rate acceleration and elevated SCRs, demonstrating the successful generation of an unconditioned response (Figure 20). Importantly, responses to the CSs confirmed successful fear conditioning on subjective and physiological levels. CS ratings of fear, arousal, and negative valence for the aversive CS+ (compared with the neutral CS+ and the CS-) increased during fear acquisition and decreased during extinction training (Figure 21). As expected, the aversive CS+ (relative to the neutral CS+) evoked heart rate

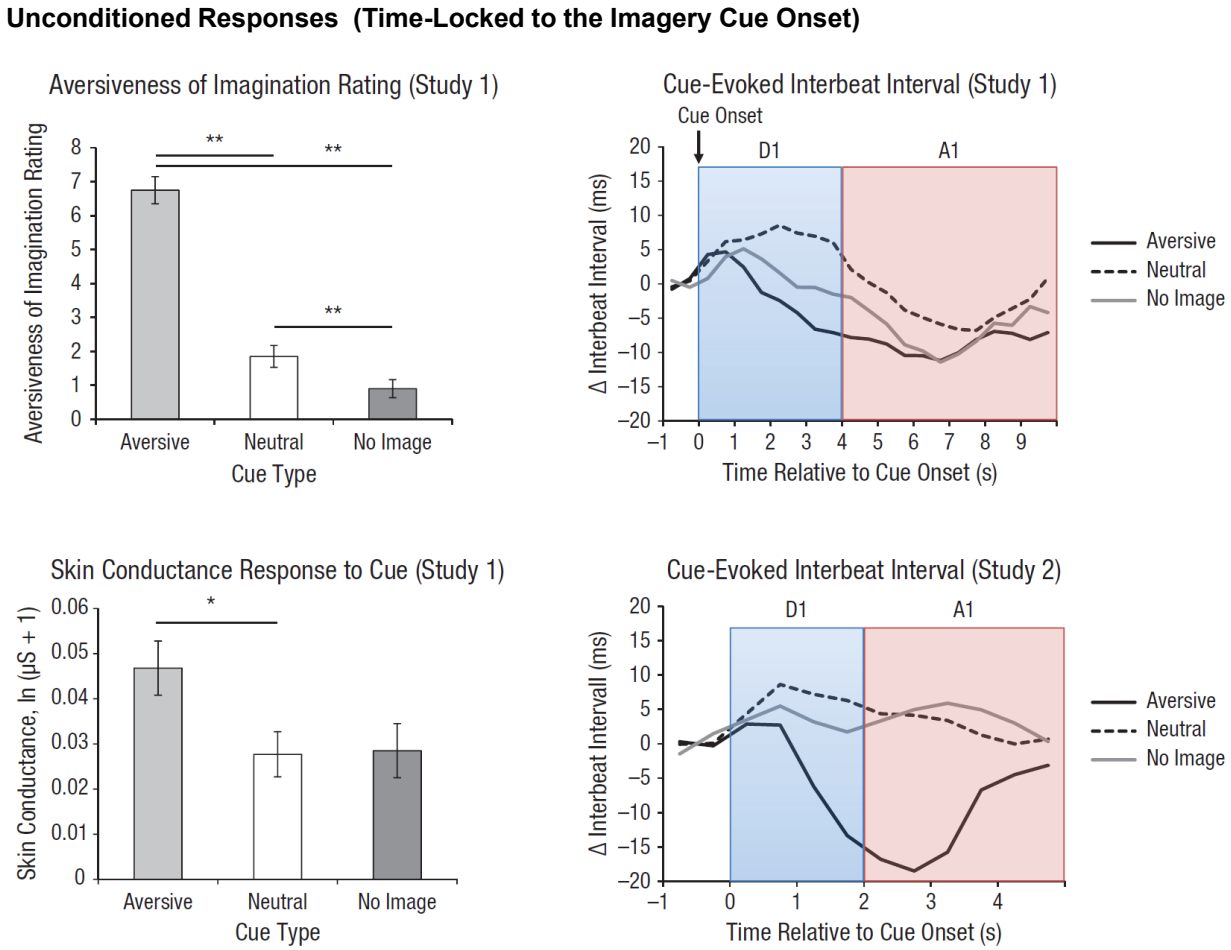


Figure 20. Unconditioned responses evoked by the aversive imagery (i.e., responses time-locked to the geometric imagery cues). The mean unpleasantness rating of mental images in the first study is shown in the upper left panel for responses to each of the three cue types. Ratings were made on an 11-point Likert-type scale ranging from 0 ("not at all") to 10 ("extremely unpleasant"). Mean skin conductance response (SCR) to each cue type in the first study is shown in the lower left panel. Error bars show repeated measures standard errors of the mean (Masson & Loftus, 2003), and asterisks indicate significant differences between cue types (* $p < .05$, ** $p < .001$). Mean evoked heart interbeat interval is shown for both studies (right panels), separately for each cue type during the deceleration time window (D1) and the acceleration time window (A1). Figure republished from Mueller, Sperl, & Panitz (2019).

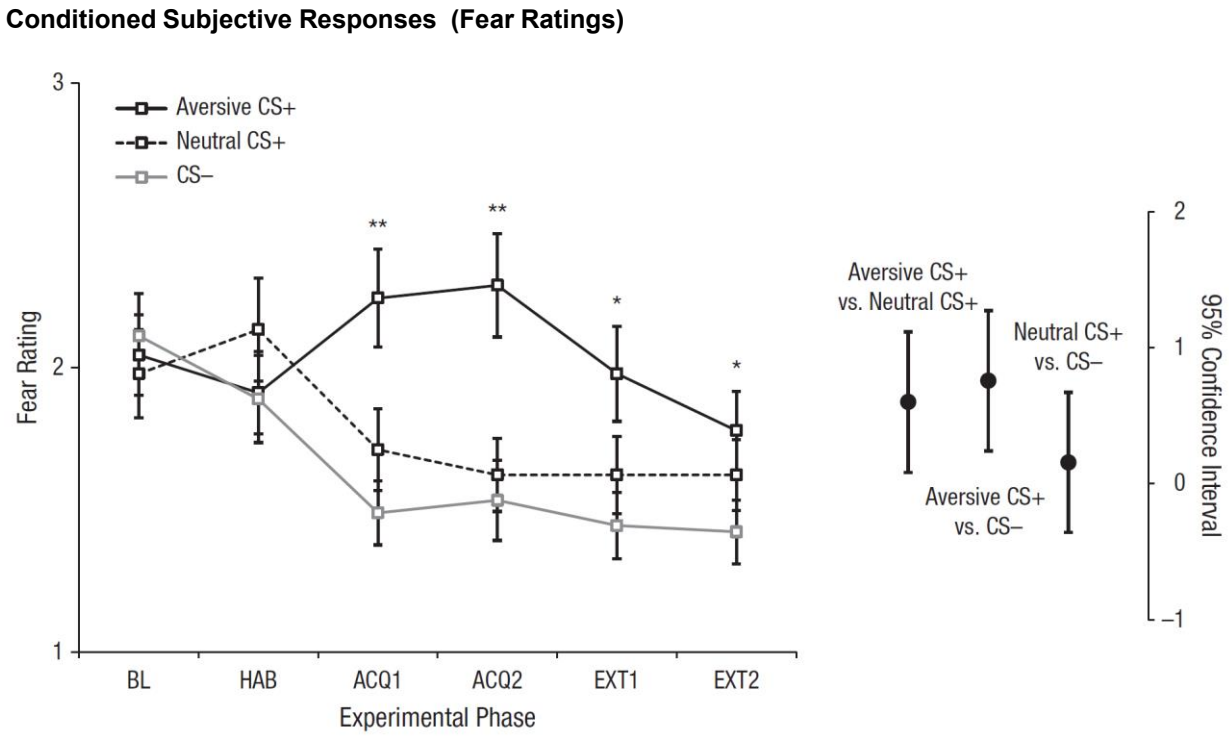


Figure 21. Fear ratings (conditioned subjective responses) in the first study described in manuscript 2. The graph on the left shows participants' mean ratings of experienced fear (5-point Likert scale, ranging from 1 = "not fearful" to 5 = "very fearful") when viewing each CS type during baseline (BL), habituation (HAB), the first acquisition block (ACQ1), the second acquisition block (ACQ2), the first extinction block (EXT1), and the second extinction block (EXT2). Error bars show repeated measures standard errors of the mean (Masson & Loftus, 2003). * $p < .05$, ** $p < .001$. On the right, 95% confidence intervals are shown for the between-conditions differences during acquisition (collapsed across ACQ1 and ACQ2; Cumming, 2014). Figure republished from Mueller, Sperl, & Panitz (2019).

deceleration, consistent with successful fear conditioning (fear-conditioned bradycardia, Figure 22A). We also observed relative heart rate deceleration for the CS- (associated with the no-imagery cue) compared with the neutral CS+ (associated with the neutral imagery). This effect can be explained by previous observations suggesting that imagery tasks evoke cardiac acceleration (Vrana & Lang, 1990). We did not find fear conditioning effects for SCRs (see chapter 5.2).

In the second study that is reported in manuscript 2, we used an imagined US that is closer to physical stimuli used in common fear conditioning studies. Electric shocks are among the most frequently used USs in animal and human fear conditioning research (see manuscript 1 of this thesis). Thus, participants were instructed to imagine receiving a strong *electric shock* on the forearm (aversive US) or receiving a mild *vibration* on the forearm (neutral US). Similarly to the first study, we also used a no-imagery condition (no-US). Note that a physical shock was *never* administered during the entire experiment. Participants only received information about an unpleasant electric stimulation in the corresponding imagery instruction.

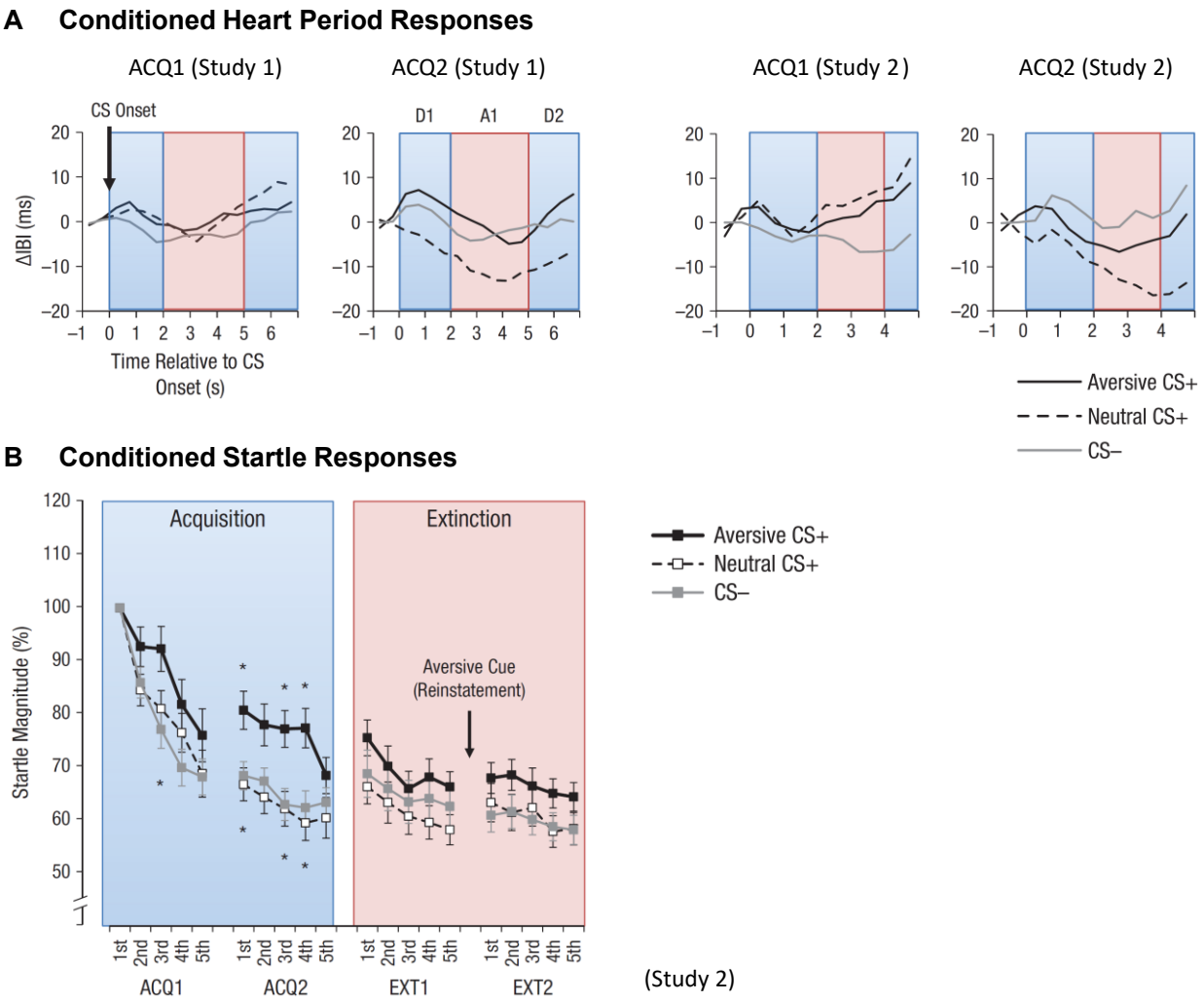


Figure 22. Conditioned physiological responses. (A) Mean CS-evoked interbeat interval (IBI, data from both studies) during the deceleration time windows (D1 and D2) and the acceleration time window (A1), separately for the first (ACQ1) and second (ACQ2) acquisition block. The aversive CS+ (compared with the neutral CS+) evoked relative cardiac deceleration in ACQ2. (B) Because of the good signal-to-noise ratio, startle data were analyzed at the single-trial level in the second study (Sevenster et al., 2013; Soeter & Kindt, 2010, 2012a). Mean normalized single-trial eyelid startle responses to the 85 dB noise burst are displayed as a function of the CS type. The aversive cue was presented between the first (EXT1) and second (EXT2) extinction blocks to test for reinstatement of fear. Error bars show repeated measures standard errors of the mean (Masson & Loftus, 2003). Asterisks (*) above the lines indicate significant differences between the aversive CS+ and the neutral CS+, and asterisks (*) below the lines indicate significant differences between the aversive CS+ and the CS- ($p < .05$). Figure republished from Mueller, Sperl, & Panitz (2019).

Replicating our findings from the first study of manuscript 2, fear conditioning with an imagined shock US was successful on subjective (affective ratings) and cardiovascular levels. Heart period results for the second study (imagined shock) were similar to findings from the first study (imagined thumbtack). The aversive imagery evoked cardiac acceleration (unconditioned response, Figure 20), while the aversive CS+ was associated with cardiac deceleration (conditioned response, Figure 22A). This response pattern of heart period changes closely

resembles findings from fear conditioning studies with physical USs (Lipp, 2006). As explained in more detail in chapter 1.5, physical USs (e.g., electric shocks) evoke heart rate acceleration (see Figure 9D; Ginsberg & Thysell, 1966; Lipp, 2006; Lipp & Vaitl, 1990; Vila et al., 2007), while CS+ versus CS- is typically followed by heart rate deceleration (see Figure 9C; Deane & Zeaman, 1958; Gruss et al., 2016; Notterman et al., 1952; Panitz et al., 2015; Thigpen et al., 2017; Yin et al., 2018). Although this divergence may seem paradoxical at first glance, it is highly adaptive with regard to the functional meaning of unconditioned versus conditioned responses (Davis & Lang, 2003; Löw et al., 2015; Obrist, 1976), which is further explained in chapter 3.4 (discussion section). Corresponding with the first study, we did not find fear-conditioned SCRs, indicating that sympathetic arousal did not differ between CS types. In the second study described in manuscript 2, we also studied fear-potentiated startle responses (Blumenthal et al., 2005), which is thought to be a relatively pure measure of stimulus valence (Hamm & Weike, 2005; Vrana & Lang, 1990). In contrast to SCRs, startle responses better reflect processes outside of conscious control (Hamm & Weike, 2005; Oyarzún et al., 2019; Sevenster et al., 2014; Weike et al., 2007). Thus, the fear-potentiated startle technique also allowed us to rule out demand effects (see chapter 1.5 of this thesis). As expected, startle probes (white noise bursts) during the aversive CS+ (compared with the neutral CS+ and the CS-) evoked significantly enhanced eyelid startle responses, which diminished during extinction training (see Figure 22B).

Remarkably, we showed in two independent samples that *de novo* fear conditioning (as measured with affective ratings, fear-conditioned bradycardia, and fear-potentiated startle) is possible with aversive mental images as USs only. Our results may have critical implications for etiological models of anxiety disorders. We demonstrated that associative fear learning is possible in the total absence of any *physical* aversive experience, and without observational, vicarious, and instructional learning. Our findings suggest that the development of pathological fear does *not* require physical traumatic experiences. Manuscript 2 expands traditional conceptualizations derived from learning theories, such as (see Figure 15 in chapter 1.7) the model by Ressler and colleagues (Bowers & Ressler, 2015; Maddox et al., 2019; Parsons & Ressler, 2013; Ross et al., 2017). These results are of particular relevance in light of the recent popularity of imagery-based interventions in clinical psychology (Blackwell, 2021; Hendrikx et al., 2021; Maloney et al., 2019; Pearson et al., 2015; Rijkeboer et al., 2020; Romano et al., 2020; Strachan et al., 2020). Fear conditioning with imagined USs may thereby help to connect imagery-based approaches with more traditional behavioristic concepts (Mertens et al., 2020). Changing dysfunctional imageries of social embarrassment and suffocation, for example, may be promising approaches to refine cognitive-behavioral interventions in social anxiety disorder and agoraphobia, respectively.

2.3 Manuscript 3:
Learning Dynamics of Electrophysiological Brain Signals During Human Fear Conditioning

Sperl, M. F. J., Wroblewski, A., Mueller, M., Straube, B., & Mueller, E. M. (2021). Learning dynamics of electrophysiological brain signals during human fear conditioning. *NeuroImage*, *226*, 117569. https://doi.org/10.1016/j.neuroimage.2020.117569

Open Data and **Open Materials** available online at *Zenodo*:
https://doi.org/10.5281/zenodo.4294603

Scientific Recognition:
I received an **RTG 2271 Poster Award** for this project at the 2019 retreat of the **Research Training Group (RTG) 2271 "Breaking Expectations"** in Hirschegg, Austria.

The EEG methodology relies on averaging a large number of trials to ensure a sufficiently high signal-to-noise ratio (Huffmeijer et al., 2014; Steinberg et al., 2013). It has already been stressed in the introduction for manuscript 1 that this technical constraint needs to be considered when choosing an appropriate US type for fear conditioning research. If participants habituate (due to the large number of trial repetitions), conditioned responses become already extinguished during the acquisition training. Beyond that, this averaging procedure can cause further problems that are highly relevant for EEG fear conditioning research. Averaging across many trials implies that *all* trials of the acquisition stage are weighted equally for all subsequent statistical analyses. Although this averaging procedure is usually applied in most EEG fear conditioning studies (Miskovic & Keil, 2012), it is in marked contrast to theoretical knowledge from learning theory and empirical observations (e.g., obtained from animal work or human fMRI studies) about temporal characteristics of underlying brain mechanisms.

The Rescorla–Wagner model (Rescorla & Wagner, 1972) is among the most influential formal models to explain fear conditioning by emphasizing changes in associative strength between the CS+ and the US. According to this model, a discrepancy between US *expectancy* (associated with the CS+/-) and the actual occurrence of the US is critical for successful fear conditioning and extinction. Notably, this expectancy is changing from early to late fear acquisition and extinction trials. During early acquisition trials, for example, the US occurs "unexpectedly" and cannot be predicted reliably by the CS+ yet. This "positive prediction error" increases the CS-US association. Conversely, during early extinction training trials, the absence of the US produces a negative prediction error and thus reduces the association between the CS+ and the US. Although learning

may be defined as a *change in neural activity* due to experience (Ferreira de Sá et al., 2019), averaging across a high number of acquisition or extinction trials (as it is typically done in EEG research) completely ignores this theoretical knowledge. Following assumptions of the Rescorla–Wagner model (Rescorla & Wagner, 1972) and other more recent associative learning models (Tzovara et al., 2018), the visualization in Figure 3 (see chapter 1.1) assumes a linear increase in conditioned responses during fear acquisition training. However, it is often not further probed if neural responses really follow this linear increase, or if changes could be better described by other growth curves (e.g., quadratic or cubic trends). With a few exceptions (e.g., Ferreira de Sá et al., 2019; Liu, Keil, & Ding, 2012), EEG research has mostly neglected temporal dynamics across trials, due to methodological constraints (signal-to-noise ratio, see above).

As explained in chapter 1.2, the amygdala plays a key role in fear conditioning. It is well known from animal research and human fMRI studies that the amygdala shows a rapid habituation pattern over time (see chapter 1.3). Activity from the amygdala is difficult to capture with EEG, due to the deep location and small size of this brain area (Tzovara et al., 2019). Nevertheless, we assume that activity from the amygdala contributes to ERP components that are relevant for fear conditioning and can be measured with scalp EEG recordings (Bunford et al., 2018; Levita et al., 2015; Vuilleumier, 2009). If a large number of EEG trials is averaged, brain activations with rapid habituation patterns are likely to be overlooked.

Given these neurophysiological (e.g., habituation of amygdala responses) and theoretical (e.g., implications of the Rescorla–Wagner model) considerations, there is a great need to develop experimental designs for EEG research that are (a) sensitive to habituation-prone neural responses and (b) allow one to unravel how threat signatures evolve and change during learning. In manuscript 3 of this thesis, we developed and validated a new sequential-set fear conditioning paradigm to address these issues. This paradigm (see Figure 23) comprises three successive acquisition training phases, each with a novel CS+/CS- set. Each of the three sets is denoted with a subscript ($CS+_1$/$CS-_1$; $CS+_2$/$CS-_2$; $CS+_3$/$CS-_3$). Each CS set consisted of two different faces with neutral expressions on different background colors that were used as CS+ and CS-, respectively. For the first acquisition training (ACQ1), the first CS set ($CS+_1$/$CS-_1$) was used. During the second acquisition training (ACQ2), the second CS set ($CS+_2$/$CS-_2$) was presented. Finally, during the third acquisition training (ACQ3), the third CS set ($CS+_3$/$CS-_3$) was shown. Participants were instructed about the CS-US contingency prior to acquisition training (Hollandt et al., 2020). Participants were told that the CS+ face would be paired with an aversive electric shock US, but they did not receive information about the reinforcement rate (Mertens et al., 2018).

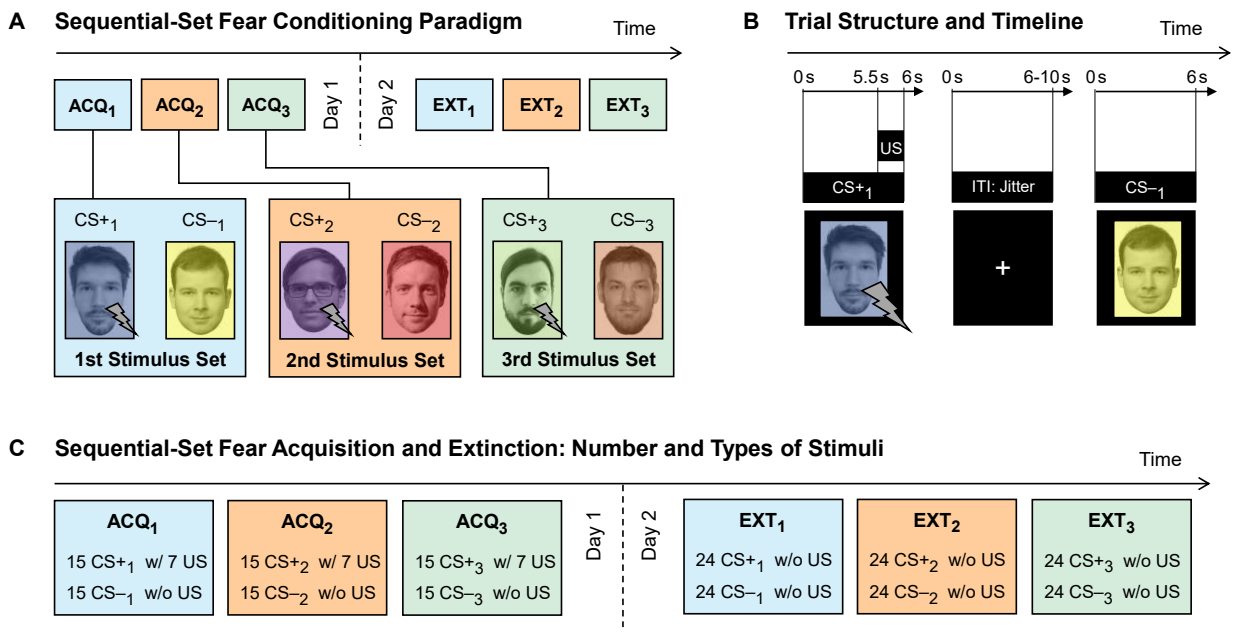


Figure 23. Sequential-set fear conditioning paradigm used in manuscript 3. (A) Participants underwent three successive acquisition training phases (ACQ1, ACQ2, and ACQ3), each with a novel conditioned stimulus (CS+/CS-) set (differently tinted neutral faces). For example, the $CS+_1$/$CS-_1$ stimulus set was used during the first acquisition training (ACQ1). Approximately 24 hours after fear conditioning, subjects underwent sequential-set extinction, which consisted of three successive extinction training phases (EXT1, EXT2, and EXT3), each with the corresponding CS+/CS- set. (B) Trial structure and timeline for a single CS trial. All CSs were presented for 6 s, followed by a jittered 6–10 s intertrial interval (ITI). During acquisition training, CS+ were paired with an aversive electric shock unconditioned stimulus (US). The delivery of the US started 500 ms before the CS offset. (C) The number and types of stimuli presented during the experimental phases. During three acquisition training phases, $CS+_1$, $CS+_2$, and $CS+_3$ were reinforced (reinforcement rate of 47%) with an aversive US ("w/"), while $CS-_1$, $CS-_2$, and $CS-_3$ were never paired with a US ("w/o"). Note: Due to licensing restrictions, the original stimulus material that was used in the present study cannot be published. To illustrate the paradigm, panels A and B contain comparable stimuli with faces of the authors of manuscript 3 and their colleagues. Figure republished from Sperl et al. (2021).

To validate our new sequential-set fear conditioning paradigm, we assessed affective CS ratings and physiological data. CS-evoked changes in skin conductance and heart rate are displayed in Figure 9 in chapter 1.5. In each of the three CS+/- sets, the respective CS+ compared with the corresponding CS- evoked elevated SCRs and relative cardiac deceleration. As the paradigm was specifically designed for EEG research, we were particularly interested in modulations of CS-evoked ERPs. It has already been outlined in chapter 1.4 that, in previous fear conditioning studies, researchers analyzed different ERP components and concrete time windows varied enormously among studies. These circumstances were especially problematic for the validation of our *new* sequential-set fear conditioning paradigm, as it was difficult to derive periods for ERP analysis from previous studies. Time windows with different ERP modulations for CS+ compared with

CS- (in particular during early periods) could thus be easily overlooked. To circumvent this problem, we followed a data-driven approach. Specifically, we applied the so-called topographic analysis of variance (TANOVA) method (Koenig & Melie-García, 2009; Murray et al., 2008). This technique identifies a continuous period of the ERP trace during which topographic maps differ between CS+ and CS- (Gianotti et al., 2008). This procedure does not make any *a priori* assumptions with regard to time windows or electrode sites (Michel & Murray, 2012; Murray et al., 2008). Differences between maps can be related to amplitude strength (i.e., amount of simultaneously active sources) and topography (i.e., location/orientation of active sources). We used the TANOVA method as implemented in the Randomization Graphical User Interface (RAGU) software package (Habermann et al., 2018; Koenig et al., 2011; Koenig & Melie-García, 2009). To compute the TANOVA, trials from all three CS+/- sets were averaged. TANOVAs were performed separately for acquisition and extinction stages.

For the fear acquisition stage on day 1, the TANOVA identified three periods with continuously significant ERP map differences for CS+ compared with CS- (see Figure 24A): (a) 33–60 ms, (b) 108–200 ms, and (c) 468–820 ms. During the 33–60 ms time window, we observed more negative ERP amplitudes for CS+ versus CS- at centro-parietal channels (Figure 24B), suggesting a rapid detection of threat cues and privileged signal transmission already during early processing stages. This finding is in line with previous studies showing that fear conditioning modulates early visual cortical processing (Hintze et al., 2014; Mueller & Pizzagalli, 2016; Stolarova et al., 2006; Thigpen et al., 2017). It has been suggested that subcortical brain regions (i.e., amygdaloid nuclei) have close connections to perceptual areas and may gate threat processing in these areas during initial learning (Chen et al., 2009; Freese & Amaral, 2005; Pourtois et al., 2013; Rotshtein et al., 2010; Vuilleumier et al., 2004). Over the course of further learning trials, plastic changes may then lead to facilitated perception in neurons of the primary visual cortex (Keil et al., 2007; McTeague et al., 2015; Thigpen et al., 2017). The visual C1 wave, which is generated mainly in the primary visual cortex, is assumed to be one of the earliest ERP components (Clark et al., 1994; Jeffreys & Axford, 1972; Rauss et al., 2011). Although our effects during the 33–60 ms period may be related to C1 processes, genuine C1 waves, as described in the literature, typically start slightly later, with an onset latency around 40–60 ms and peaks between 80 and 100 ms (Luck, 2014). Exploratory follow-up comparisons within each of the three CS+/- sets (e.g., $CS+_1$ versus $CS-_1$) were not significant, consistent with previous observations that a massive number of trials are necessary to detect short-latency ERP fear conditioning effects (Miskovic & Keil, 2012; Stolarova et al., 2006; Thigpen et al., 2017).

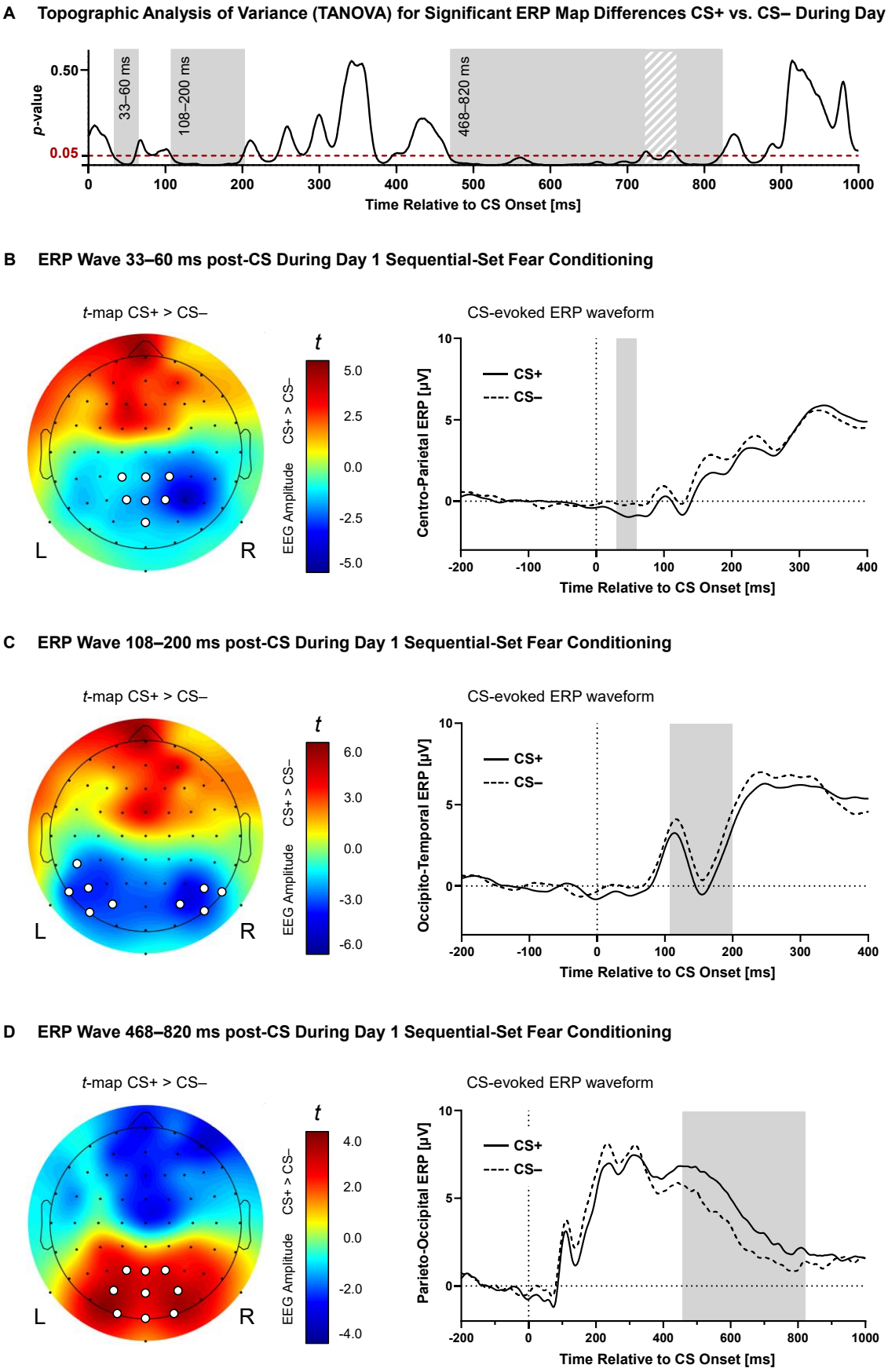


Figure 24. Event-related potential (ERP) responses evoked by CS+ compared with CS- during day 1 sequential-set fear conditioning. (A) The topographic analysis of variance (TANOVA) indicated that

topographic maps were significantly different for CS+ compared with CS- during the 33–60 ms, 108–200 ms, and 468–820 ms periods (i.e., *p*s ≤ .05, gray-shaded areas). The last time window was interrupted by a short period (719–730 ms) with .05 ≤ *p* ≤ .08 (shaded in gray and white). (B) During 33–60 ms, the ERP amplitude was significantly more negative for CS+ compared with CS- at centro-parietal electrode sites (left panel). To visualize ERP waveforms (right panel), the electrode sites CP1, CPz, CP2, P1, Pz, P2, and POz were averaged (channels are shown as white dots in the *t*-map). (C) During 108–200 ms, the ERP amplitude was significantly more negative at occipito-temporal channels for CS+ versus CS-. The ANOVA on occipito-temporal ERP amplitudes yielded a significant *Contingency* (i.e., CS+ versus CS-) × *Channel* interaction, and significantly more negative amplitudes for CS+ compared with CS- occurred at CP5, P7, P5, PO7, and PO3 over the left hemisphere, as well as at P8, P6, PO8, and PO4 over the right hemisphere (channels are shown as white dots in the *t*-map). To visualize ERP waveforms (right panel), electrodes with significant effects were averaged. (D) During 468–820 ms, the ERP amplitude was significantly more positive at parieto-occipital channels for CS+ versus CS- (left panel). To visualize ERP waveforms (right panel), the electrode sites P1, Pz, P2, PO3, POz, PO4, O1, Oz, and O2 were averaged (channels are shown as white dots in the *t*-map, significant effects could be confirmed at all electrode sites). The gray-shaded areas in panels B, C, and D indicate the measurement windows for ERP amplitudes. "L" = left hemisphere, "R" = right hemisphere. Figure republished from Sperl et al. (2021).

Furthermore, the acquisition TANOVA revealed more negative ERPs for CS+ compared with CS- between 108 and 200 ms after CS onset over occipito-temporal electrode sites (Figure 24C), indicating privileged threat processing in extrastriate regions (Clark & Hillyard, 1996; Linkenkaer-Hansen et al., 1998). Significant differences between ERPs following CS+ and CS- during this period could even be confirmed within each of the three CS+/- stimulus sets (i.e., $CS+_1$ versus $CS-_1$, $CS+_2$ versus $CS-_2$, and $CS+_3$ versus $CS-_3$), emphasizing the robustness of this finding (Figure 25A). This relatively broad period contains the typical ERP latencies of the P1 and N170 components (Desjardins & Segalowitz, 2013). More negative ERP amplitudes for CS+ versus CS- could thus be interpreted as reduced P1 amplitudes, as elevated N170 amplitudes, or as a combination of both processes. On the one hand, attenuated P1 responses would be in line with a study by Liu, Keil, & Ding (2012), who found decreased P1 amplitudes for well-trained CS+/CS- stimulus pairs. This finding would be consistent with the prediction error theory of attention (Pearce & Hall, 1980), suggesting that less attention is required as soon as the US gets predicted by the CS+ (Liu, Keil, & Ding, 2012). On the other hand, more negative ERP amplitudes during this time window would also be in agreement with the N170 literature. Some electromagnetic studies suggest larger N170 amplitudes for CS+ compared with CS- faces (Camfield et al., 2016; Levita et al., 2015; Mueller & Pizzagalli, 2016; Pizzagalli et al., 2003; Steinberg et al., 2012; Watters et al., 2018), although there have been contradictory findings (Stolarova et al., 2006; Stolz et al., 2019).

Finally, the TANOVA for the acquisition stage showed a larger positivity for CS+ compared with CS- between 468 and 820 ms at parieto-occipital sensors (Figure 24D). This result is highly

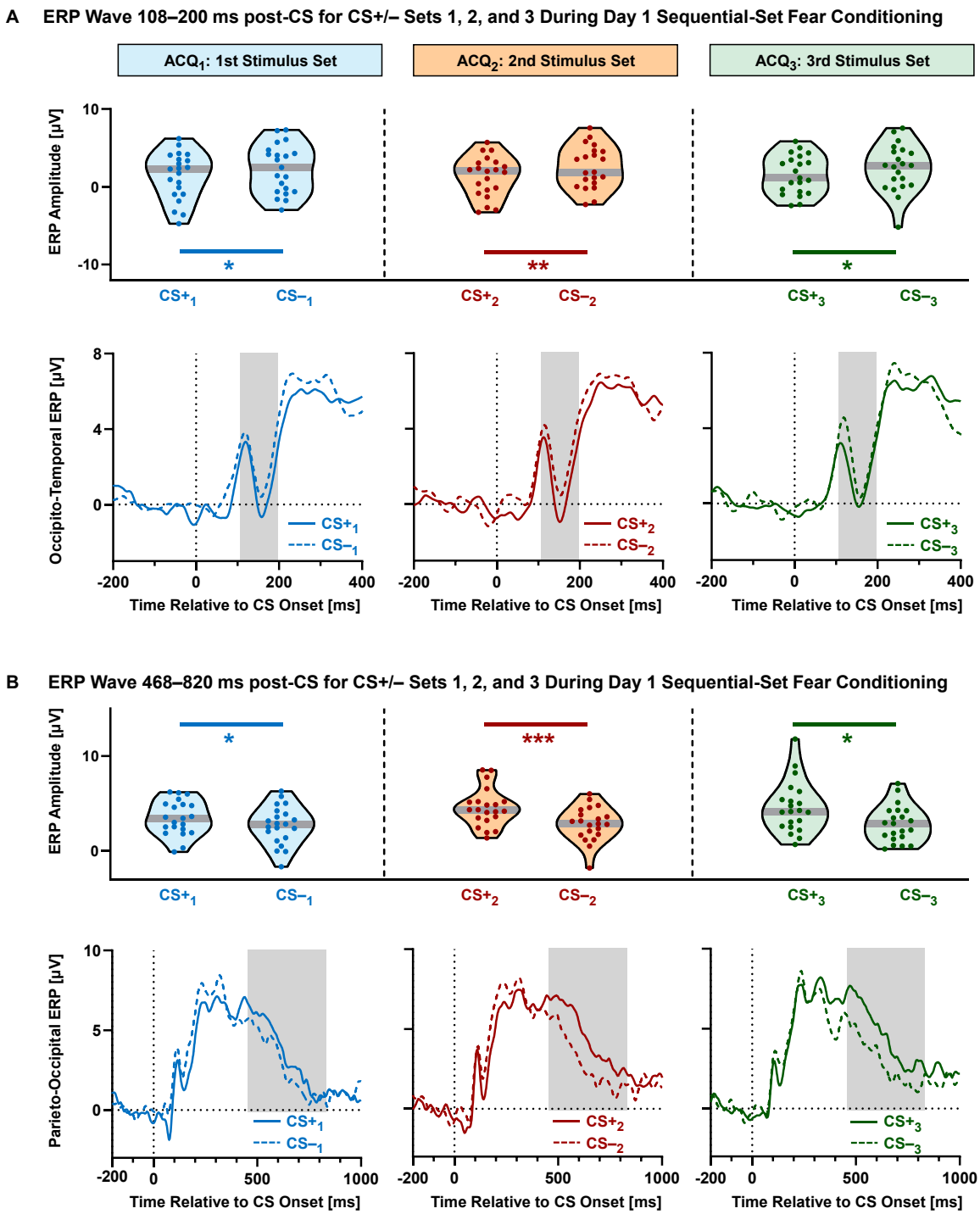


Figure 25. Event-related potential (ERP) responses evoked by CS+ compared with CS- during day 1 sequential-set fear conditioning were comparable across the three stimulus sets. (A) The occipito-temporal ERP amplitude during 108–200 ms after CS onset was significantly more negative for CS+ compared with CS- in all three CS sets. (B) The parieto-occipital ERP amplitude during 468–820 ms time-locked to the CS onset was significantly more positive for CS+ compared with CS- in all three CS sets. In the upper panels, violin plots display the frequency distribution of the ERP data (averaged across channels that are marked with white dots in Figure 24). Individual data points (i.e., participants) are superimposed on the violin plot, and the median is displayed as a gray horizontal line. In the lower panels, the time series of CS-evoked changes in voltage (relative to baseline) are shown. Gray-shaded areas indicate time windows for statistical analyses. *** $p \leq .001$, ** $p \leq .01$, * $p \leq .05$. Figure republished from Sperl et al. (2021).

consistent with previous studies confirming that fear conditioning potentiates LPP responses (Bacigalupo & Luck, 2018; Ferreira de Sá et al., 2019; Panitz et al., 2015; Pastor et al., 2015; Pavlov & Kotchoubey, 2019; Seligowski et al., 2018; Stolz et al., 2019). Similarly to ERP effects during the 108–200 ms period, LPP effects were also significant within each of the three CS+/- sets (Figure 25B). LPP activity appears to be generated in an extended network of cortical and subcortical brain regions (Liu, Huang et al., 2012). Elevated LPP amplitudes represent elaborative processing due to stimulus significance (Hajcak & Foti, 2020), going along with the activation of a neural "defensive system" (Bradley, 2009). Modulations of LPP amplitudes indicate neurophysiological processes that are linked to perceptual, motivational, and motor signaling (Wieser & Keil, 2020).

Most importantly, our experimental design allowed us to uncover the *learning curve* of EEG responses during the three time windows described above (see Figure 26). Therefore, the acquisition stage was split into smaller sub-blocks of five trials each. Four pre-acquisition trials that were shown prior to acquisition training were also averaged. This approach provides the possibility to assess changes in neural responding from early to late acquisition trials. Collapsing across CS+/- sets ensures (a) an adequate signal-to-noise ratio (averaging *across* CS+/- sets), while (b) creating the opportunity to examine changes over time that are related to learning (averaging only across *a few* trials *within* each CS+/ set).

Notably, differential ERP responses during the 33–60 ms period increased throughout fear acquisition trials and followed a linear growth curve (Figure 26A). Effect sizes showed a stepwise increase until a large conditioned response was visible during the last acquisition trials. Remarkably, this gradual increase in short-latency ERP signatures closely mirrors the above-mentioned hypothesis of subsequent plasticity in primary visual neurons. A similar pattern emerged for the 108–200 ms period (Figure 26B). Conditioned responses increased from early to late fear acquisition, and this increase could be best described by a linear trend. After a sharp rise during the first five acquisition trials, effect sizes reached a plateau with a rather smaller subsequent increase. Similarly, conditioned responses during the 468–820 ms period showed a linear increase, with constantly growing effect sizes (Figures 26C and 26E).

We computed a separate TANOVA for extinction training on day 2. We expected a large conditioned response during early trials that should decline toward later extinction trials. Thus, the extinction TANOVA was calculated for the average ERP during *early* extinction training (i.e., the first eight extinction trials from all three CS+/- sets). The extinction TANOVA revealed significant differences between ERP maps for CS+ versus CS- from 460 to 730 ms post-CS – a period that is

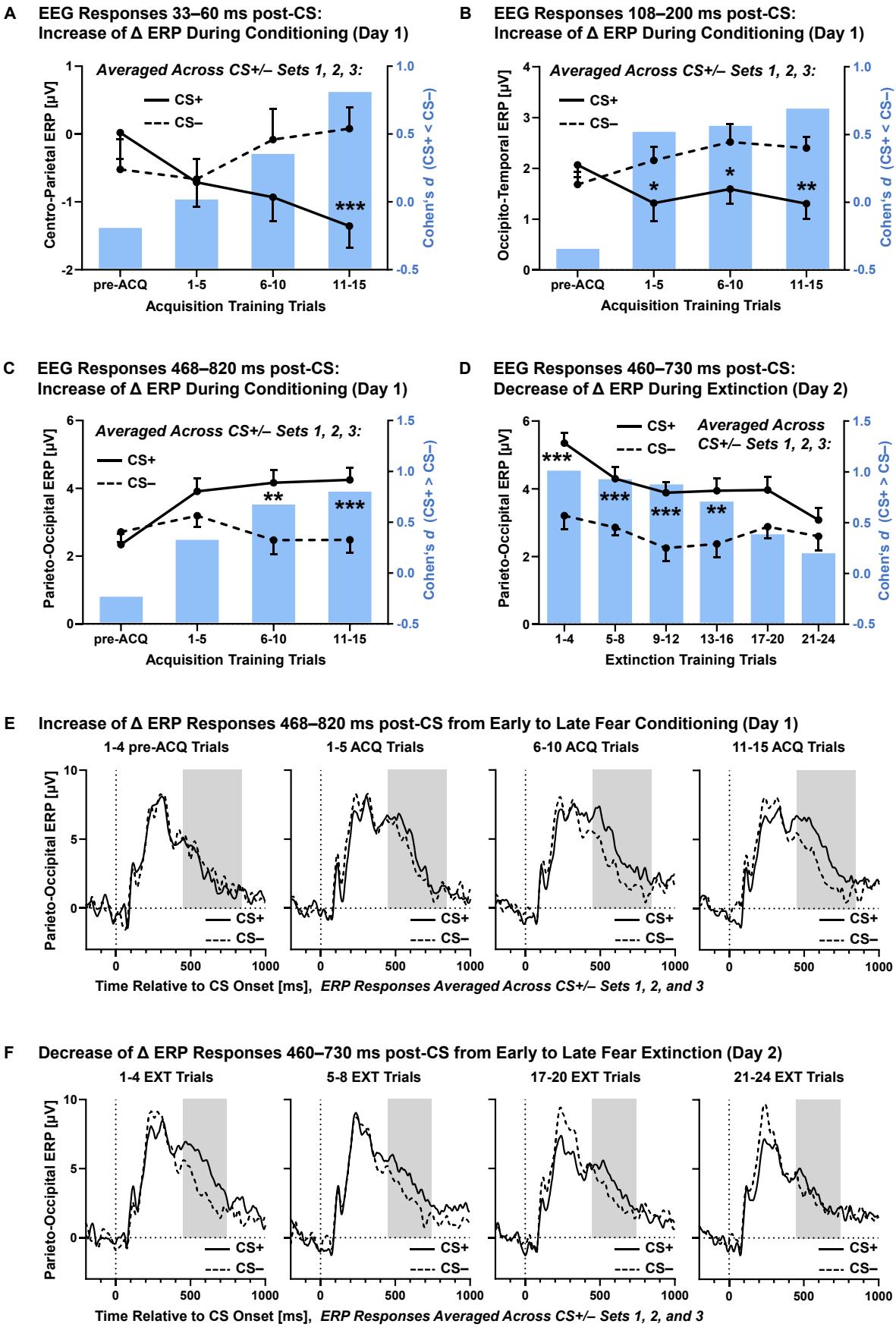


Figure 26. To detect changes over time, the acquisition and extinction training phases were split into smaller sub-blocks of five (acquisition) or four (pre-acquisition and extinction) trials each. Averaging

across trials from all three conditioned stimulus (CS+/CS-) sets allows studying the increase and decrease in threat-related modulation of event-related potentials (ΔERPs; CS+ versus CS-) during fear conditioning and extinction, respectively. Conditioned EEG responses during the (A) 33–60 ms, (B) 108–200 ms, and (C, E) 468–820 ms periods increased from early to late fear conditioning (day 1). Conversely, conditioned responses during (D, F) 460–730 ms decreased from early to late extinction (day 2). Line charts (A–D) show the mean voltage for CS+ and CS- for each sub-block (± within-subject *SEM*, O'Brien and Cousineau, 2014). Blue bars indicate how effect sizes (Cohen's *d*, plotted on the right *y*-axis) for conditioned electrocortical responses increased during fear conditioning (A–C) and decreased during fear extinction (D). *** $p \leq .001$, ** $p \leq .01$, * $p \leq .05$. Figure republished from Sperl et al. (2021).

very similar to the late-latency LPP time window of day 1. Similarly to day 1 acquisition, we observed a sustained positive deflection at parieto-occipital channels that was significantly larger for CS+ compared with CS-. The extinction TANOVA did not reach significance during short- and mid-latency time windows. To elucidate temporal dynamics during extinction training, each extinction training phase was split into six sub-blocks of four trials each, and these sub-blocks were averaged across the three CS+/- sets to triple the relative amount of trials. As expected, differential late-latency ERP responses during the 460–730 ms period gradually diminished from early to late extinction training trials (Figures 26D and 26F). Conditioned responses decreased linearly, which was accompanied by a successive decline of effect sizes.

Manuscript 1 of the present thesis (see chapter 2.1) showed that the use of electric shock USs in EEG conditioning studies (which typically have many trials) can be problematic – especially, when shocks are administered in standard intensities ("highly annoying but not painful"). If the shock intensity is too low, unconditioned responses can show a strong habituation pattern across trials. As a consequence of this finding from manuscript 1, we used a relatively high shock intensity in the study described in manuscript 3, with the goal to avoid habituation to the US. To probe explicitly whether unconditioned responses declined over time, we also analyzed peripheral and EEG responses to the US. Unconditioned peripheral responses (i.e., responses to the US) were similar across CS+/- sets and consisted of heightened SCRs and cardiac acceleration (data are shown in Figure 9 in chapter 1.5), mirroring the responses to imagined USs that are presented in manuscript 2 (see chapter 2.2). Furthermore, the US evoked similarly strong ERP responses in each of the three CS+/- sets. Specifically, the US was followed by a sharp fronto-central negative deflection from 50 to 200 ms (see Figures 27A and 27C) and a broader centro-parietal positive deflection from 200 to 350 ms (see Figures 27B and 27D), which is consistent with prior research (Christmann et al., 2007; Deguchi et al., 1996; Kenntner-Mabiala et al., 2008; Miltner et al., 1989; Nelson et al., 2015; Wang et al., 2014; Wang & Tian, 2018; Yamaguchi & Knight, 1991). The

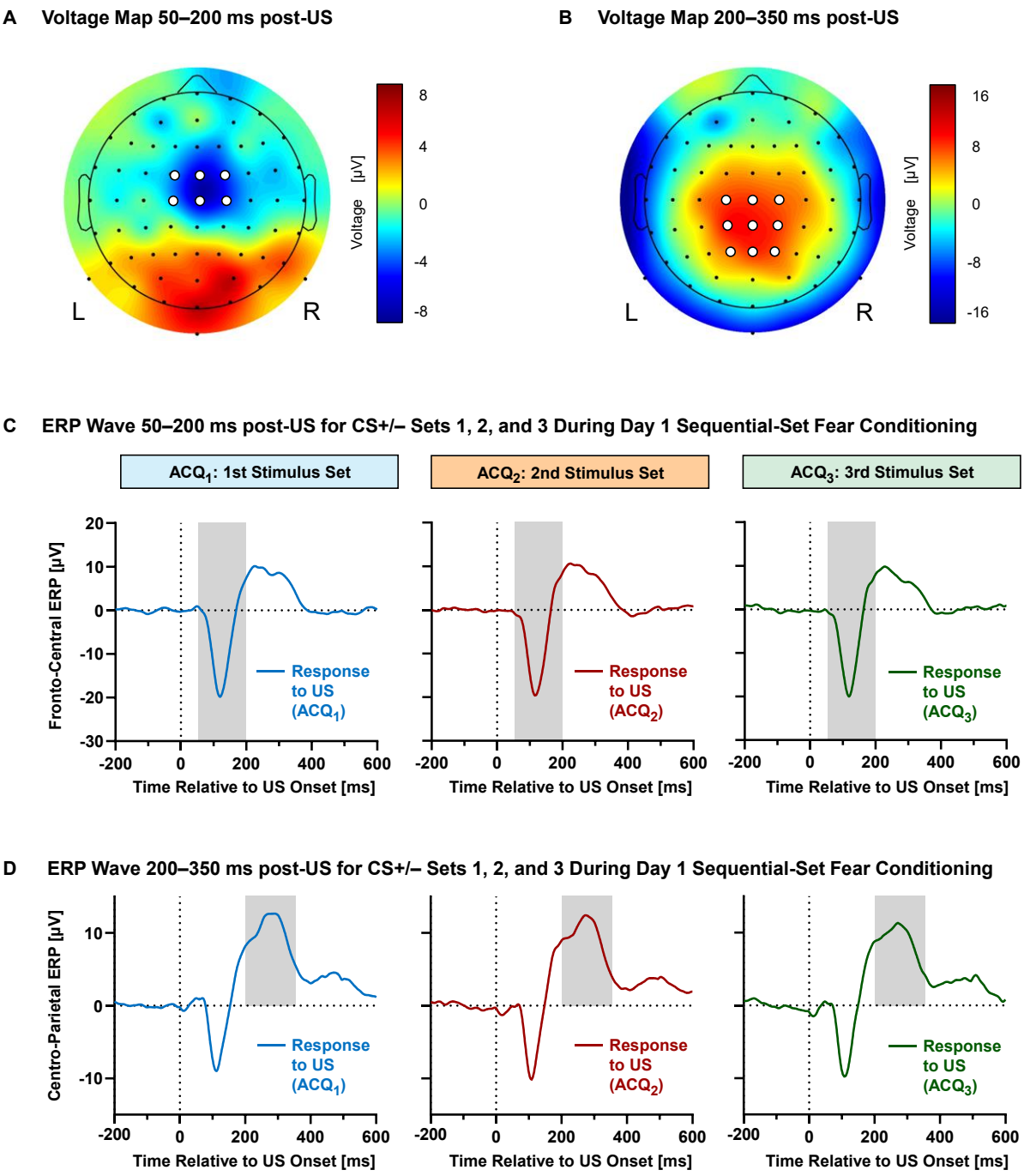


Figure 27. Event-related potential (ERP) responses evoked by the US during day 1 sequential-set fear conditioning were comparable across the three acquisition training phases for CS set 1, set 2, and set 3. The US was associated (A) with a large fronto-central negative deflection from 50 to 200 ms, followed by a (B) centro-parietal positive deflection from 200 to 350 ms after stimulus onset (gray-shaded areas). (C) To visualize ERP waveforms during the 50–200 ms period, the electrode sites FC1, FCz, FC2, C1, Cz, and C2 were averaged (channels are shown as white dots in the voltage map). (D) To visualize ERP waveforms during the 200–350 ms period, the electrode sites C1, Cz, C2, CP1, CPz, CP2, P1, Pz, and P2 were averaged (channels are shown as white dots in the voltage map). "L" = left hemisphere, "R" = right hemisphere. Figure republished from Sperl et al. (2021).

earlier component has been linked to somatosensory processing (Apkarian et al., 2005; Christmann et al., 2007). This negativity was largest at midline channels and extended to rather right-hemispheric electrode sites, resembling previous findings of enhanced ERP amplitudes at channels that are *contralateral* to the hand at which electric shocks were applied (Wang et al., 2014). The later positivity, which overlaps with the typical P3 time window (Yamaguchi & Knight, 1991), has been associated with rather top-down modulated evaluation (Christmann et al., 2007; Kenntner-Mabiala et al., 2008; Valentini et al., 2013).

In conclusion, we developed a new *sequential-set* fear conditioning paradigm that is particularly well suited for EEG research. While learning dynamics have mostly been ignored in previous EEG studies on fear conditioning, our design is a powerful tool to unravel spatio-temporal dynamics of neural threat processes. Averaging across CS+/CS- sets allows one to illustrate how differential ERP responses during short-, mid-, and long-latency periods gradually rise during fear acquisition and vanish throughout extinction learning.

2.4 Manuscript 4:
Alpha-2 Adrenoreceptor Antagonist Yohimbine Potentiates Consolidation of Conditioned Fear

Sperl, M. F. J., Panitz, C., Skoluda, N., Nater, U. M., Pizzagalli, D. A., Hermann, C., & Mueller, E. M. (submitted). Alpha-2 adrenoreceptor antagonist yohimbine potentiates consolidation of conditioned fear. Submitted to *Neuropsychopharmacology*.

Open Data and **Open Materials** will be available online at *Zenodo* after acceptance.

Scientific Recognition:
I received an **SPR Poster Award** from the **Society for Psychophysiological Research (SPR)** for this project, awarded at the 57th Annual Meeting 2017 at the Hofburg Vienna, Austria.

In the manuscripts 1, 2, and 3, we aimed to answer the following questions: Which US types are suitable for fear conditioning studies with many trials (as it is typically the case in EEG research)? Are physical CS-US pairings really necessary for successful fear conditioning? How is it possible to uncover the learning curve of EEG signatures during fear acquisition and extinction? In these studies, we addressed important methodological issues in fear conditioning research. Manuscript 2 is of particular relevance to bridge between basic conditioning studies and etiological models of pathological fear, given that many patients do not recall an aversive or traumatic event that might be causal or critical for the development of anxiety disorders. Furthermore, manuscript 3 translates knowledge about temporal dynamics during threat learning from animal models (where suitable methods are available to record neural activity with high spatial and temporal precision) to the human realm.

As outlined in chapter 1.6, it is well known that catecholamines (especially noradrenaline) modulate the consolidation of emotional memories. Elevated noradrenergic activity during states of elevated arousal (e.g., in the aftermath of a traumatic event) has been discussed to promote hyperconsolidation of aversive experiences, which might be an important etiological factor for the development of pathological fear. Conversely, pharmacologically boosted noradrenergic transmission during or after conducting an exposure therapy session could be a promising approach to facilitate the efficacy of therapeutic interventions. However, previous pharmacological studies in patient samples (e.g., those using the noradrenergic substance yohimbine) have revealed mixed effects on the outcome of exposure interventions (see chapter 1.6). Thus, we aimed to explore how yohimbine affects fear consolidation and extinction learning in an established and well-controlled fear conditioning and extinction paradigm (see Figure 28).

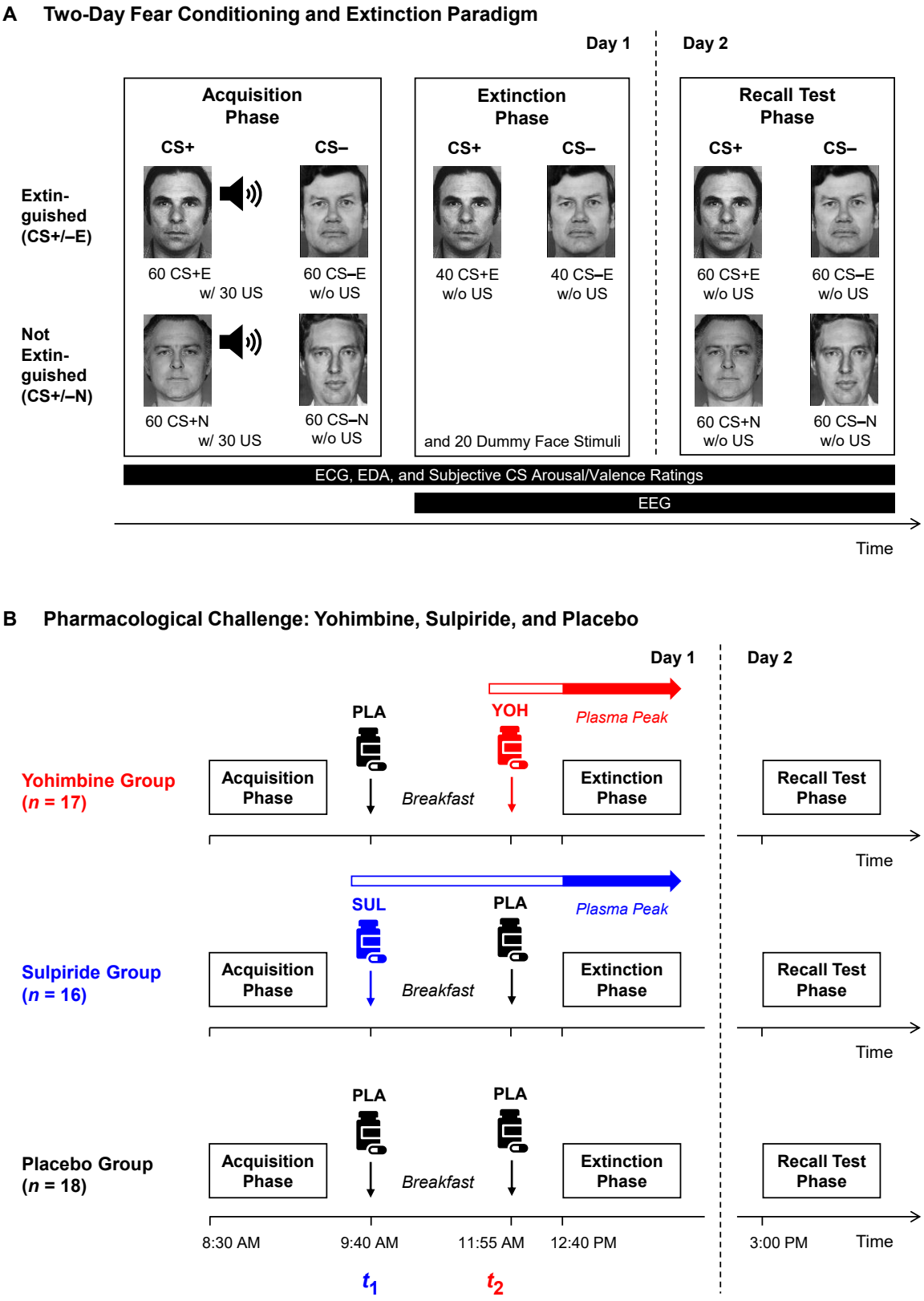


Figure 28. Experimental fear conditioning and extinction paradigm used in the study described in manuscript 4. (A) Stimulus types and number of presentations during the three experimental phases. (B) Pharmacological challenge. Between fear acquisition and extinction stages, participants received an oral dose of either 10 mg of yohimbine HCl (YOH), 200 mg of sulpiride (SUL), or a placebo pill (PLA). Note that both substances differ in the time they take to reach peak plasma concentration. Thus, sulpiride

was administered at 9:40 AM (= t_1), and yohimbine at 11:55 AM (= t_2), to ensure that participants from both experimental groups reached peak plasma levels at a similar point. To guarantee successful blinding for experimenters and participants, each participant received two capsules (e.g., participants in the sulpiride group received the active substance sulpiride at t_1 and a placebo pill at t_2; participants in the placebo group received two placebo pills).

We applied a two-day experimental design that has been successfully used in prior EEG fear conditioning research (Mueller et al., 2014; Mueller & Pizzagalli, 2016; Panitz et al., 2015) and is similar to the paradigm of manuscript 1. Instead of stimuli from the Lundqvist set (Lundqvist et al., 1998), neutral faces from the Ekman series (Ekman & Friesen, 1976) served as CSs. On the first day, participants underwent fear acquisition and extinction training (see Figure 28A). Two CS+ (CS+E, CS+N) and two CS- (CS-E, CS-N) were shown during acquisition. Both CS+ were paired with an aversive US. Based on our results from manuscript 1, an unpleasant white noise burst served as US. During extinction training, only one of the two CS+ (the "extinguished" CS+, CS+E) and one of the two CS- (the "extinguished" CS-, CS-E) were presented, to extinguish conditioned responses to these stimuli (CS+E versus CS-E). The "nonextinguished" CSs (CS+N, CS-N) and the US were not presented during extinction training (see manuscript 1). During a recall test stage on the second day, all stimuli (i.e., CS+E, CS-E, CS+N, CS-N) were shown without any US presentation. Comparing differential responses for nonextinguished (CS+N versus CS-N) and extinguished (CS+E versus CS-E) stimuli allowed us to differentiate between fear recall (nonextinguished CSs) and extinction recall (extinguished CSs).

To assess catecholaminergic effects on fear consolidation and fear extinction, participants received, in a double-blind manner, the noradrenergic substance yohimbine or placebo between acquisition and extinction training (see Figure 28B). As expected, yohimbine administration increased salivary α-amylase activity (sAA, see Figure 29), which is an indicator for central noradrenaline release (Ditzen et al., 2014; Ehlert et al., 2006; Nater & Rohleder, 2009). We only tested male participants, as neural effects of yohimbine seem to be sex dependent (Schwabe et al., 2013) and fear/extinction recall is modulated by estrogen levels (Merz et al., 2018; see also chapter 3.3 in the discussion section). As explained in chapter 1.6, yohimbine does not only act at noradrenergic receptors; yohimbine also has antagonist properties at dopaminergic D2 receptors (Holmes & Quirk, 2010; Millan et al., 2000; Scatton et al., 1980). To further disentangle noradrenergic and dopaminergic mechanisms on fear/extinction memory processes, a third experimental group received the dopaminergic substance sulpiride (see Figure 28B).

Affective CS ratings and peripheral physiological data (SCRs, heart period) confirmed successful fear acquisition and extinction on day 1. Importantly, heart period data for day 2

revealed elevated fear recall for the yohimbine group compared with the placebo and sulpiride groups. Only participants in the yohimbine group showed cardiac deceleration for the nonextinguished CS+N compared with CS-N. This contrast reflects successful recall of conditioned fear that has not yet been extinguished (see Figure 30). Conversely, cardiac responses to the extinguished CS+E and CS-E did not differ. There were no significant effects in the placebo and sulpiride groups (neither for CS+N versus CS-N, nor for CS+E versus CS-E). Taken together, yohimbine administration on the first day induced heightened recall of fear-conditioned bradycardia on the following day.

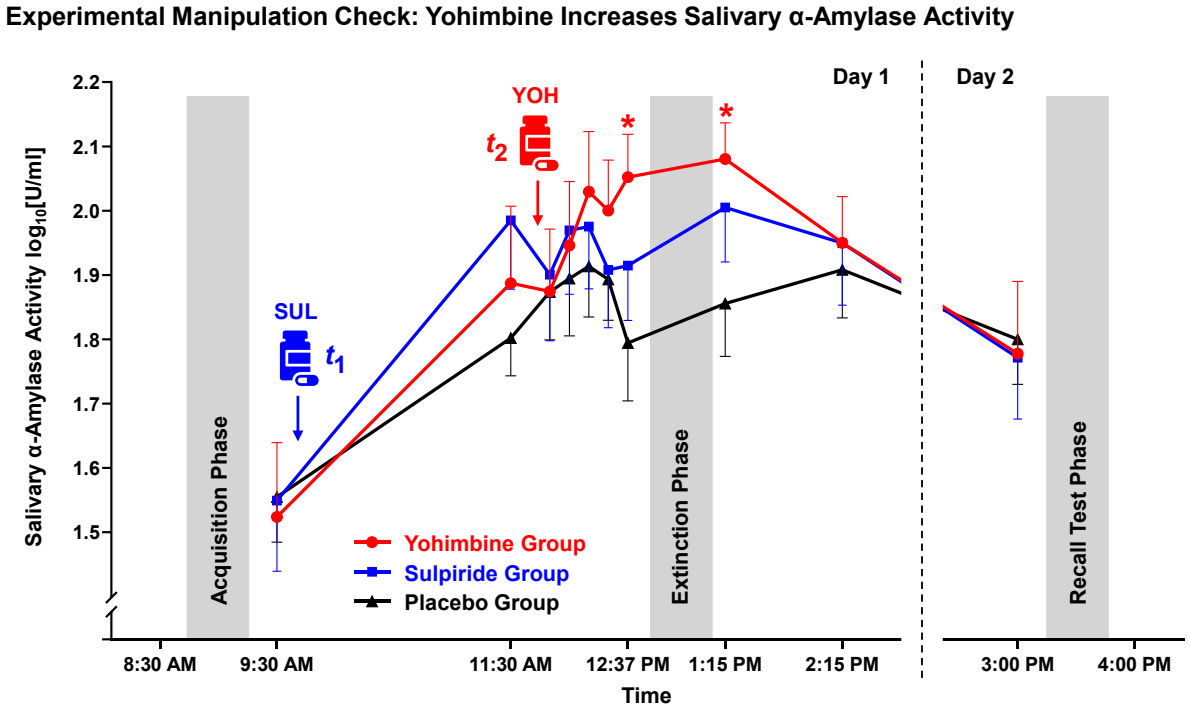


Figure 29. Salivary α-amylase activity (sAA) was assessed to confirm the successful influence of yohimbine (YOH) on central noradrenaline release. Compared with the placebo, yohimbine administration was associated with significantly elevated sAA activity directly before and after extinction training. * $p \leq .05$.

Remarkably, EEG responses during the typical time windows of two ERP components closely mirrored yohimbine effects on fear-conditioned bradycardia. We were specifically interested in the face-sensitive N170 component and the LPP, as we found reliable fear conditioning effects for these latencies in manuscript 3 (see chapter 2.3). During the N170 period, differential ERP responses for nonextinguished CSs were significantly larger in the yohimbine group (compared with the placebo and sulpiride groups). Specifically, the CS+N versus CS-N evoked larger N170 amplitudes for participants who received yohimbine (see Figure 31). There was also a significant

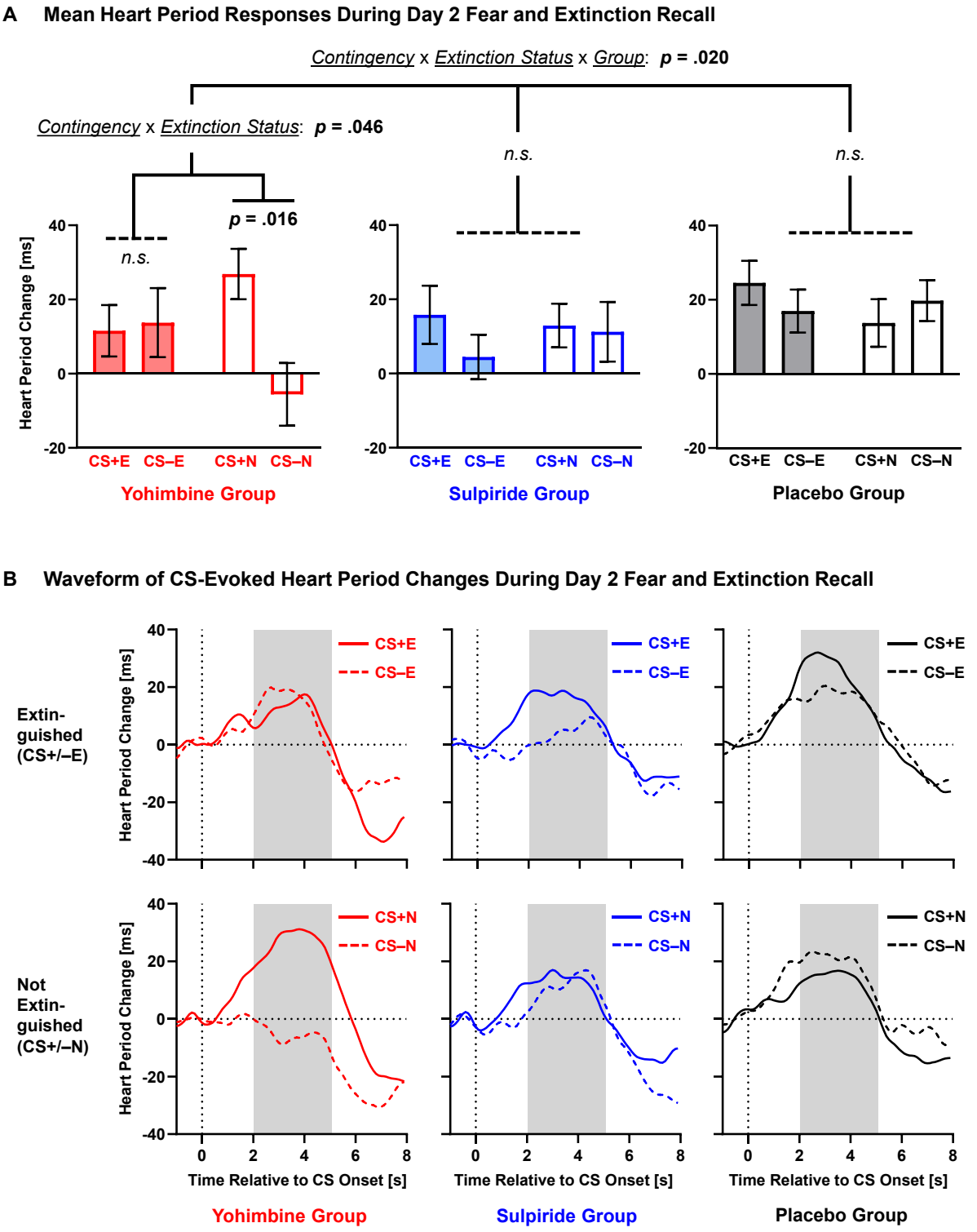


Figure 30. Fear-conditioned bradycardia (mean heart period change 2–5 s post-CS) during day 2 recall. (A) The ANOVA for CS-evoked heart period changes revealed a significant *Contingency* (CS+/-) × *Extinction Status* (extinguished/nonextinguished, E/N) × *Group* interaction. Only the yohimbine group showed stronger cardiac deceleration for the nonextinguished CS+N compared with CS-N, indicating enhanced recall of fear-conditioned bradycardia. Mean (± within-subjects *SEM*, adjusted within each group; O'Brien & Cousineau, 2014) heart period changes after CS onset are displayed. (B) The waveform of CS-evoked heart period changes is shown for extinguished (CS+E, CS-E; upper panels) and nonextinguished (CS+N; CS-N; lower panels) stimuli, separately for the yohimbine (left panels), sulpiride (middle panels), and placebo (right panels) groups.

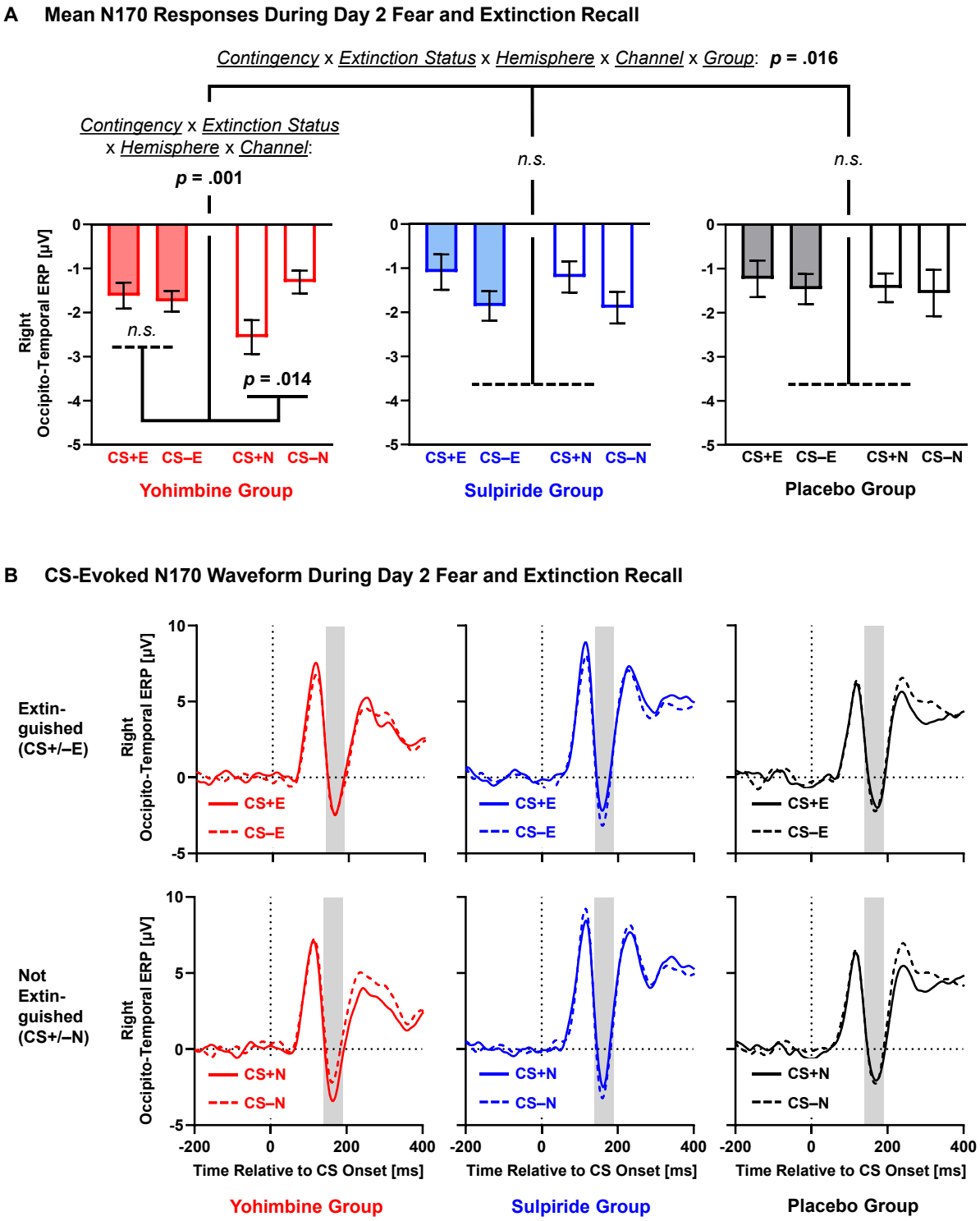


Figure 31. CS-evoked N170 component during day 2 recall. The ANOVA on mean amplitudes (145–185 ms post-CS) yielded a significant *Contingency* (CS+/-) × *Extinction Status* (extinguished/nonextinguished, E/N) × *Hemisphere* × *Channel* × *Group* interaction. Only the yohimbine group showed significantly larger (i.e., more negative) N170 amplitudes for the nonextinguished CS+N compared with CS-N, and effects were restricted to the channels TP10, P8, and P010 over the right hemisphere. To illustrate (A) mean voltage changes (± within-subjects *SEM*, adjusted within each group; O'Brien & Cousineau, 2014) and (B) event-related potential (ERP) waveforms, the electrode sites TP10, P8, and P010 were averaged. The EEG data were referenced against Cz, as this central reference highlights better the N170 at occipito-temporal channels (Joyce & Rossion, 2005).

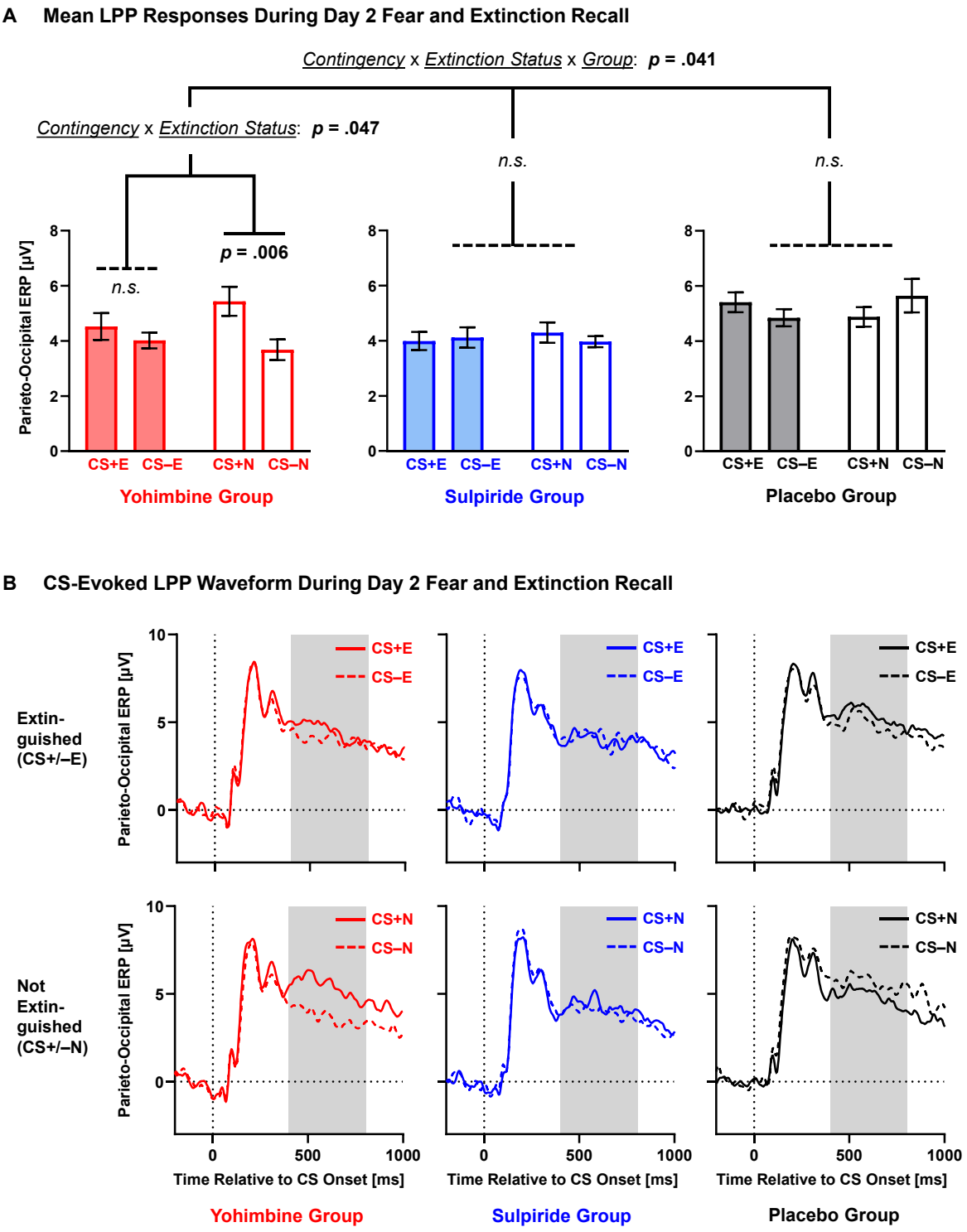


Figure 32. CS-evoked late positive potential (LPP) component during day 2 recall. The ANOVA on mean amplitudes (400–800 ms post-CS) yielded a significant *Contingency* (CS+/-) × *Extinction Status* (extinguished/nonextinguished, E/N) × *Group* interaction. Only the yohimbine group showed significantly larger (more positive) LPP amplitudes for the nonextinguished CS+N compared with CS-N. As there was no significant interaction with the *Channel* factor, all parieto-occipital channels (P1, Pz, P2, PO3, POz, PO4, O1, Oz, and O2) were averaged to illustrate (A) mean voltage changes (± within-subjects *SEM*, adjusted within each group; O'Brien & Cousineau, 2014) and (B) event-related potential (ERP) waveforms. The EEG was referenced to the average of TP9 and TP10 (mastoids), which is consistent with the majority of LPP studies (Hajcak et al., 2012; Hajcak & Foti, 2020). The mastoid reference allows emotion-related LPP modulations to be better highlighted (Hajcak et al., 2012).

interaction with the *Hemisphere* factor (i.e., left/right brain hemisphere). Significant effects could only be confirmed at *right* hemispheric electrode sites. This observation converges with prior findings on N170 lateralization effects (Eimer, 2011; Levita et al., 2015; Pizzagalli et al., 2003; Rossion & Jacques, 2012; but: Camfield et al., 2016), in accordance with the hypothesized advantage of the right brain hemisphere for face (Frässle et al., 2016) and danger-related emotion processing (Gainotti, 2019). Effects during the N170 time window were specific for the comparison of nonextinguished stimuli (CS+N versus CS-N; "fear recall"), but were absent for extinguished stimuli (CS+E versus CS-E; "extinction recall"). We did not observe any significant N170 effects in the sulpiride and yohimbine groups.

Similarly to EEG responses during the N170 period, yohimbine administration on the first day led to greater conditioned responses during the LPP time window on day 2. One day after fear acquisition and extinction, we found elevated differential (CS+ versus CS-) LPP amplitudes for nonextinguished stimuli in the yohimbine group (see Figure 32). The nonextinguished CS+N compared with the CS-N evoked significantly larger LPP amplitudes in the yohimbine group only. Consistent with successful extinction recall, responses to the extinguished CS+E and CS-E did not differ. Complementing the N170 results reported above, we did not find differential ERP responses (for both nonextinguished and extinguished stimuli) in the placebo and sulpiride groups. The functional meaning of the LPP component (which was also studied in manuscripts 3 and 5) has been discussed in more detail in chapter 1.4. Briefly, LPP modulations are associated with sustained attention, alongside the activation of cortico-limbic brain networks (Liu, Huang et al., 2012) that are related to perceptual, motivational, and motor processes (Hajcak & Foti, 2020; Wieser & Keil, 2020). LPP can thus be conceptualized as an index of stimulus significance (Hajcak & Foti, 2020). ERP amplitudes during this time window are particularly enhanced for emotionally engaging stimuli (Cuthbert et al., 2000).

In conclusion, noradrenergic (but not dopaminergic) stimulation after fear acquisition facilitated fear expression on the following day. The absence of sulpiride effects, together with elevated salivary α-amylase activity for the yohimbine group, suggests that yohimbine facilitated fear consolidation through heightened noradrenaline release (see chapter 4.4 for details). Our findings may have critical implications for neurobiological models on the pathogenesis of pathological fear, which is further discussed in chapter 3.2 in the discussion section.

2.5 Manuscript 5:
Fearfulness, Neuroticism/Anxiety, and COMT Val158Met in Long-Term Fear Conditioning and Extinction

Panitz, C., **Sperl, M. F. J.**, Hennig, J., Klucken, T., Hermann, C., & Mueller, E. M. (2018). Fearfulness, neuroticism/anxiety, and COMT Val158Met in long-term fear conditioning and extinction. *Neurobiology of Learning and Memory*, *155*, 7–20. https://doi.org/10.1016/j.nlm.2018.06.001

In manuscript 4 (chapter 2.4), we demonstrated facilitated fear consolidation after the pharmacological administration of yohimbine, as measured by elevated conditioned responses one day after acquisition. Yohimbine is a catecholaminergic neurotransmitter with high relevance for the consolidation of emotional memories (see chapter 1.6). In chapter 1.6, different research strategies to investigate catecholaminergic processes have been discussed (see Figure 11). In addition to pharmacological studies, the comparison of participants with different catecholamine-related genotypes is another approach to gain insights about noradrenergic and dopaminergic mechanisms.

In manuscript 5, we followed this genetic strategy. We recruited $N = 383$ individuals, who were screened for the *COMT* Val158Met genotype. Based on this pool of interested individuals, participants were invited for our study based on the *COMT* Val158Met polymorphism, with the goal to create genotype-balanced groups. Three genotype-related groups ($n = 32$ Val/Val, $n = 31$ Val/Met, $n = 30$ Met/Met) underwent a two-day fear conditioning and extinction study. As explained in chapter 1.6, the Met allele has been linked to increased prefrontal dopamine levels and seems to play a role in the etiology of pathological fear. Similarly to the study of manuscript 4, only male individuals were assessed, to control for possible interactions between sex and genotype (Risbrough et al., 2014). We used a fear conditioning paradigm similar to the designs described in manuscripts 1 and 4, which allows to differentiate between fear recall (CS+N, CS-N) and extinction recall (CS+E, CS-E) on day 2.

Importantly, fear and extinction recall on the second day was modulated by the genotype factor. With regard to fear-conditioned bradycardia (see Figure 33), Val/Val carriers showed robust relative cardiac deceleration for the nonextinguished (CS+N compared with CS-N, “fear recall”), but not for the extinguished stimulus pair (CS+E compared with CS-E, “extinction recall”). We did not observe significant effects for Val/Met and Met/Met carriers. Regarding neural EEG responses, we obtained very similar LPP results (see Figure 34). Converging with our heart period

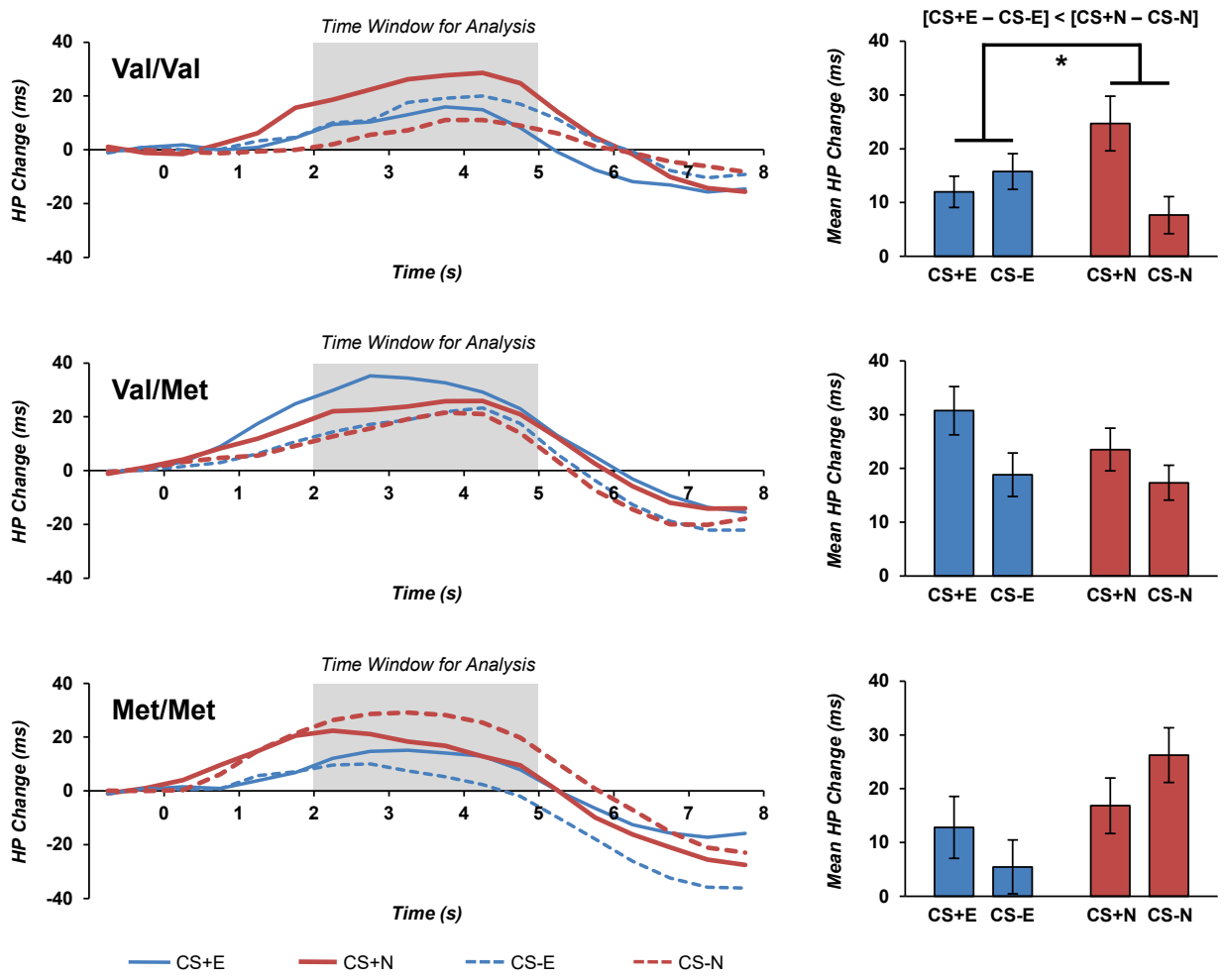


Figure 33. COMT Val158Met effects on day 2 fear-conditioned bradycardia. CS-evoked change in heart period for the different genotype groups during the first 10 artifact-free trials of the day 2 recall test. Mean magnitude within the gray box was used for statistical analyses. Error bars in the bar plots indicate *SEM* based on within-subject variance. * $p \leq .05$ for the *Contingency* (CS+/-) × *Extinction Status* (extinguished/nonextinguished, E/N) interaction. Figure republished from Panitz, Sperl et al. (2018).

findings, differential LPP amplitudes indicating fear and extinction recall also differed between genotypes. Only participants in the Val/Val group showed successful fear recall, as indicated by elevated LPP amplitudes for CS+N compared with CS-N. Conversely, there was no difference between CS+E and CS-E ("extinction recall") for Val/Val participants. In the Val/Met and Met/Met groups, no significant effects were observed for either nonextinguished (CS+N compared with CS-N) or extinguished (CS+E compared with CS-E) stimuli.

To sum up, the *COMT* Val158Met genotype predicted successful fear recall and extinction recall on the second day. Peripheral (fear-conditioned bradycardia) and central (LPP) physiological measures revealed converging results. Val/Val homozygotes were characterized by

the most adaptive physiological response pattern. On day 2, only participants in this genotype group showed robust conditioned responses to danger-predicting stimuli (i.e., stimuli that have not been extinguished on the previous day). These participants, however, did not show threat responses to extinguished stimuli (i.e., stimuli that do no longer predict danger), reflecting successful safety learning. From an evolutionary point of view, this response pattern is highly adaptive, as it allows one (a) to predict and avoid potential danger in the future ("fear recall"), but also (b) enables corrective experiences when cues are no longer associated with threat ("extinction recall").

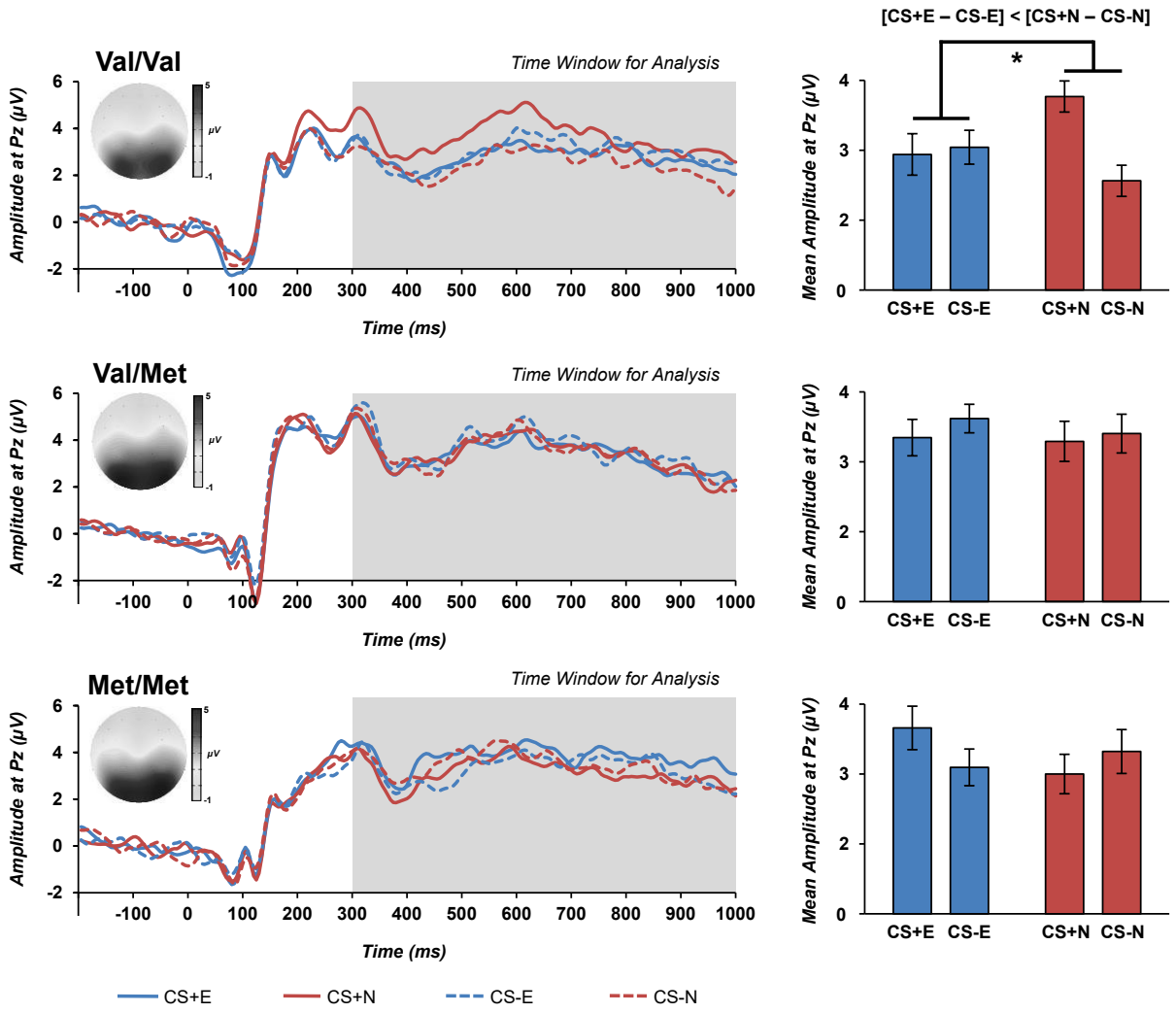


Figure 34. COMT Val158Met effects on day 2 late positive potential (LPP) responses. Event-related potential (ERP) waveforms and mean LPP amplitudes at Pz during day 2 recall test for the different genotype groups. LPP was defined as the mean amplitude from 300 to 1,000 ms (indicated by shaded areas). Topography plots show mean LPP amplitudes across all four CSs. Error bars in the bar plots indicate SEM based on within-subject variance. * $p \leq .05$ for the *Contingency* (CS+/-) × *Extinction Status* (extinguished/nonextinguished, E/N) interaction. Figure republished from Panitz, Sperl et al. (2018).

Linking our findings to catecholaminergic activity, it is important to consider that the *COMT* Val158Met polymorphism has been primarily associated with prefrontal dopamine degradation (Bilder et al., 2004; Meyer-Lindenberg & Weinberger, 2006; Yavich et al., 2007). With this in mind, it would be a straightforward interpretation to assume that relatively lower levels of prefrontal dopamine (i.e., Val/Val genotype) are associated with better consolidation of fear *and* extinction memories. We can only speculate whether this COMT-related modulation of fear/extinction consolidation is specific for dopaminergic activity, or whether it also includes noradrenergic mechanisms (see chapter 3.2 in the discussion section).

2.6 Manuscript 6:
Fear Extinction Recall Modulates Human Frontomedial Theta and Amygdala Activity

Sperl, M. F. J., Panitz, C., Rosso, I. M., Dillon, D. G., Kumar, P., Hermann, A., Whitton, A. E., Hermann, C., Pizzagalli, D. A., & Mueller, E. M. (2019). Fear extinction recall modulates human frontomedial theta and amygdala activity. *Cerebral Cortex*, *29*, 701–715. https://doi.org/10.1093/cercor/bhx353

Scientific Recognition:
I received two young scientists awards for this publication: The **Brain Products Young Scientist Award for a Distinguished Contribution in EEG Research**, which was awarded by the **German Society for Basic and Applied Psychophysiology** (Deutsche Gesellschaft für Psychophysiologie und ihre Anwendung; DGPA) and **Brain Products** (Munich); and the **WASAD Young Researcher Award**, which was awarded by the **World Association for Stress Related and Anxiety Disorders** (WASAD).

In manuscript 3 (chapter 2.3), we elucidated learning dynamics of ERP amplitudes (e.g., LPP) during fear conditioning and extinction. We further demonstrated how these ERP components are modulated by catecholaminergic substances (manuscript 4) and catecholamine-related genotypes (manuscript 5). In chapter 1.4, it has been explained in more detail that ERP analyses (i.e., EEG analyses in the time domain) represent only a certain part of the event-related EEG signal. Beyond that, frequency-based EEG analyses can add further important information about neural threat processing. For the study of manuscript 6, we assessed oscillatory theta activity during fear and extinction recall. In human participants, we aimed to elucidate neurophysiological mechanisms involved in communication between brain areas that have been proposed in the "traditional" neurobiological fear conditioning model (see Figure 4 in chapter 1.2). Our overarching goal was to bridge between findings from animal research and conclusions from human fMRI research. To this end, EEG and fMRI were recorded simultaneously, which allowed us to integrate information from both measures. Specifically, prefrontal theta oscillations can be assessed with EEG, while the detection of subcortical amygdala activity requires fMRI.

We used a two-day fear conditioning design similar to the paradigms applied in manuscripts 1, 4, and 5. On the second day, EEG and fMRI were recorded simultaneously. To disentangle responses related to fear versus extinction recall, we compared nonextinguished (CS+N versus CS-N) and extinguished (CS+E versus CS-E) stimuli. In chapter 1, it has been argued that

amygdala activation shows a rapid habituation curve over time. This is of particular relevance for manuscript 6, as the experimental design of this study contained a relatively high number of trials during day 2 recall. This large amount of trials is needed for an adequate signal-to-noise ratio of EEG recordings (see chapter 1.4). To account explicitly for amygdala activation during the fear and extinction recall stage (day 2), recall test trials were weighted with an exponentially decaying function. Parameters for this function were estimated based on the habituation curve of SCRs, as there is evidence for a correlation of temporal dynamics between SCRs and amygdala activation (see chapter 1.3).

During the recall test on day 2, differential (CS+ versus CS-) frontomedial theta power (measured at electrode Fz) was significantly larger for nonextinguished (CS+N, CS-N) compared with extinguished (CS+E, CS-E) stimuli. This pattern indicates successful fear and extinction recall (see Figure 35A). Theta power was enhanced for CS+N compared with CS-N, but did not differ between CS+E and CS-E. Altogether, these findings are consistent with prior animal (Burgos-Robles et al., 2009) and human (Mueller et al., 2014) research.

With regard to fMRI, nonextinguished (CS+N, CS-N) versus extinguished (CS+E, CS-E) stimuli were associated with significantly larger differential (CS+ versus CS-) activation in the left amygdala. Note that regressors involved in this contrast were modeled by an exponentially decaying function to account for amygdala habituation. Extending previous findings from fMRI research (e.g., Hermann et al., 2016; Phelps et al., 2004), we found increased left amygdala activation for the nonextinguished CS+N versus CS-N, while left amygdala activation was reduced for the extinguished CS+E versus CS-E (see Figure 35B).

Our overarching goal was to elucidate putative relations between EEG and fMRI data. Specifically, we aimed to bridge the gap between (a) findings on EEG theta oscillations and (b) knowledge about threat networks (e.g., the amygdala, see Figure 4 in chapter 1.2) obtained through previous fMRI studies. To integrate both measures, we computed a *fear and extinction recall (FER)* score for theta power at frontal-midline channel Fz that reflects the degree of differential modulation to nonextinguished versus extinguished CSs:

FER Day 2 Recall = (CS+N minus CS-N) minus (CS+E minus CS-E)
(based on Mueller et al., 2014)

This score was calculated for each participant. High FER scores indicate that conditioned responses during the fear/extinction recall test on day 2 were larger for nonextinguished (CS+N minus CS-N) compared with extinguished (CS+E minus CS-E) stimuli. Consequently, high FER scores can be interpreted as an indicator for both successful fear recall (larger conditioned

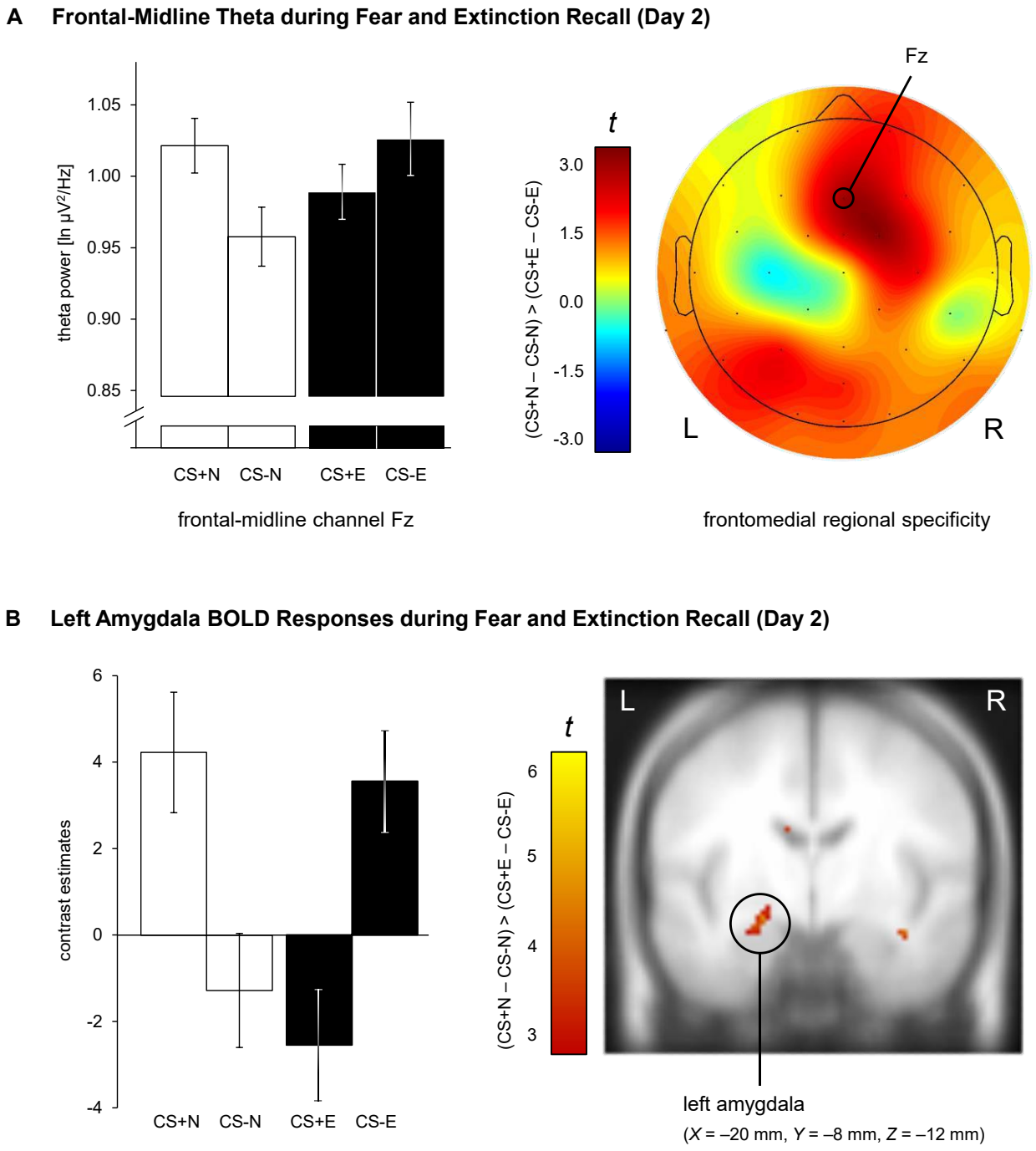


Figure 35. EEG and fMRI correlates of fear and extinction recall on day 2. (A) Differential (CS+ minus CS-) ln-transformed theta power at frontal-midline channel Fz was significantly reduced for extinguished (CS+E, CS-E) versus nonextinguished (CS+N, CS-N) stimuli (left). This effect was specific for frontomedial electrodes (right). Bar graphs show the mean theta power (± within-subject *SEM*, O'Brien & Cousineau, 2014). (B) Reduced differential amygdala responses (CS+ minus CS-) for extinguished compared with nonextinguished stimuli. Habituation of amygdala activity was modeled by an exponentially decaying function, based on habituation of skin conductance responses. For illustrative purposes, the intensity threshold was set to $p \leq .005$ (uncorrected) with a minimal cluster threshold of $k \geq 5$ contiguous significant voxels. "L" = left, "R" = right brain hemisphere. Bar graphs show the mean contrast estimates (± within-subject *SEM*, O'Brien & Cousineau, 2014) for a cluster of voxels with $p \leq .005$ (uncorrected) surrounding the peak voxel within the amygdala region of interest (ROI). Figure republished from Sperl et al. (2019).

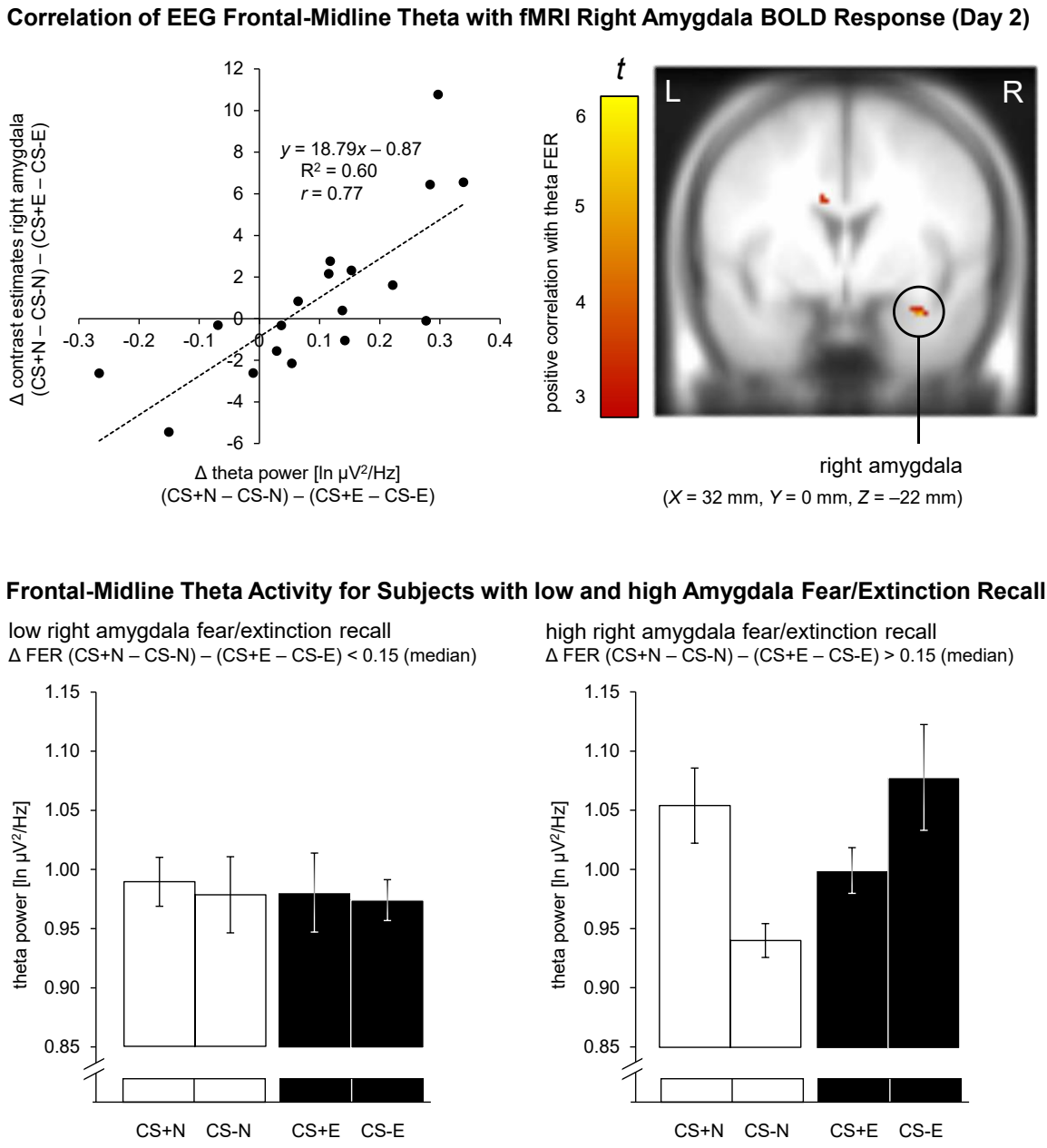


Figure 36. Integration of frontomedial (Fz) theta power (measured with electroencephalography; EEG) and right amygdala activation (measured with functional magnetic resonance imaging; fMRI) during fear and extinction recall on day 2. (A) Positive correlation of theta modulations to conditioned and extinguished fear with BOLD (blood oxygenation level dependent contrast) responses in the right amygdala. Consistent with our assumed involvement of theta oscillations in AMC-amygdala connectivity (Gilmartin et al., 2014), this correlation indicates that subjects with relatively strong amygdala activation to nonextinguished (vs. extinguished) fear stimuli are characterized by relatively strong differential frontomedial theta power. For illustrative purposes, the intensity threshold was set to $p \leq .005$ (uncorrected) with a minimal cluster threshold of $k \geq 5$ contiguous significant voxels. "L" = left, "R" = right brain hemisphere. (B) To illustrate the positive correlation, right amygdala BOLD responses for the FER contrast [(CS+N minus CS−N) minus (CS+E minus CS−E)] were compared based on median split, and theta power was assessed separately for subjects with low and high amygdala fear/extinction recall, that is, low/high FER BOLD scores (bar graphs show *M* ± within-subject *SEM*, O'Brien & Cousineau, 2014). Higher differential theta power for nonextinguished (CS+N, CS-N) versus extinguished (CS+E, CS-E) stimuli only emerged for subjects with high ($p < .001$), but not with low ($p = .929$) fear/extinction recall in the right amygdala. CS+N and CS-N: nonextinguished stimuli; CS+E and CS-E: extinguished stimuli. Figure republished from Sperl et al. (2019).

responses for nonextinguished CSs, CS+N minus CS-N) and successful extinction recall (reduced conditioned responses for extinguished CSs, CS+E minus CS-E). To integrate EEG and fMRI findings, the FER score for EEG theta activity (for each participant) was entered as covariate in a second-level simple regression fMRI analysis. This regression analysis was computed with the BOLD (blood oxygenation level dependent contrast) fMRI response for the FER contrast as criterion, as we were interested in brain activity that is modulated by fear and extinction recall. Importantly, this analysis showed a positive correlation between theta EEG FER and right amygdala BOLD FER modulation. Remarkably, 60% of the variance for the FER contrast (representing fear recall and extinction recall) was shared by right amygdala activation and theta oscillations (R^2 = .60). Our findings highlight that large FER scores for EEG theta power were associated with high fear and extinction recall as indicated by fMRI amygdala activation. This correlation pattern is visualized in Figure 36.

For the first time in human research, we demonstrated that frontomedial theta activity is associated with amygdala activation during threat processing – a finding that has been previously described in rodents (Gilmartin et al., 2014). Theta synchrony between the medial prefrontal cortex and the amygdala has been linked to better CS+/CS- discrimination in mice (Likhtik et al., 2014). Theta synchrony may be critical for bidirectional AMC-amygdala connectivity, which is further involved in sustained fear expression (Burgos-Robles et al., 2009; Gilmartin et al., 2014). EEG and MEG source-localizing studies have revealed that the AMC is the predominant generator in the human brain for frontal-midline theta rhythms (Asada et al., 1999; Mitchell et al., 2008; Mueller et al., 2014). Taken together, our results suggest altered communication between the amygdala and the prefrontal cortex during the processing of extinguished and nonextinguished fear (Likhtik & Gordon, 2014). These findings allow us to derive important implications for neurophysiological processes during threat, as debated in chapter 3.1 of the discussion section.

For the fMRI analyses above, the habituation of amygdala activation was modeled by an exponentially decaying function. To enhance comparability with other fMRI studies (e.g., Hermann et al., 2016; Milad et al., 2009, 2013; Milad, Wright et al., 2007), and to evaluate further the validity of our results, we performed an additional analysis on the first four fMRI trials (of each CS type) during day 2 recall. This analysis confirmed our results on the interplay between theta oscillations and amygdala activation described above. In addition, there was a negative correlation between EEG theta and vmPFC fMRI modulation (see Figure 6 in chapter 1.3). This observation is in line with the extinction-related inhibitory function of the vmPFC on fear expression, as proposed in the “traditional” neurobiological model (see Figure 4 in chapter 1.2 and Figure 37 in the discussion section, chapter 3.1).

3 Discussion, Integration, and Conclusion

The overarching goal of the present thesis was to elucidate electrocortical mechanisms of fear conditioning in humans, with a special focus on methodological considerations and translational perspectives. In the studies described in **manuscripts 1 and 2**, we focused on the nature of the US in fear conditioning research. These peripheral physiological studies are of particular relevance to the subsequent EEG studies, given that the study of neurophysiological fear conditioning mechanisms requires suitable US types (**manuscript 1**). It is of special significance to prevent habituation to the aversive US, as EEG requires a massive number of trials for an adequate signal-to-noise ratio.

The validity of fear conditioning paradigms as translational models for pathological fear has often been criticized. In particular, not all patients with anxiety disorders are able to recall explicit CS-US pairings. In the two studies that are reported in **manuscript 2**, we demonstrated that *imagined* (i.e., not physically presented) stimuli are entirely sufficient for successful fear conditioning. This knowledge lays the foundation that our results on neurophysiological mechanisms of threat processing (**manuscripts 3, 4, 5, and 6**) might also be relevant to explain pathological fear processes in patients that are not able to explicitly report situations with physical CS-US pairings (e.g., a dog bite for patients with dog phobia).

In the studies of the present thesis, we used EEG as a method to bridge between animal research and human studies (**manuscripts 3, 4, 5, and 6**). We developed a new sequential-set fear conditioning paradigm that is particularly well suited for EEG research (**manuscript 3**) and linked EEG substrates to fMRI findings (**manuscript 6**). Furthermore, we were specifically interested in catecholaminergic mechanisms (**manuscripts 4 and 5**). These findings might explain neurobiological factors that contribute to hyperconsolidation of aversive memories and exaggerated fear.

The findings of the present thesis highlight several methodological peculiarities in EEG fear conditioning research and advance brain models of threat processing (see chapter 3.1). Studying catecholaminergic mechanisms (noradrenaline and dopamine) provides key insight into neurophysiological processes that seem to be altered in pathological fear (chapter 3.2). Catecholaminergic models may be of great relevance to understand neurophysiological factors in the etiology of anxiety and related disorders. This knowledge may, in turn, lead to the development of new treatment strategies. Future directions are suggested in chapters 3.3 and 3.4.

3.1 The Present Thesis – Extending the "Traditional" Neurobiological Model of Fear Conditioning in Humans

With the studies described in this thesis, we have closed several gaps in human research on the neurophysiology of fear conditioning and extinction. In chapter 1.2, the essential components of the "traditional" neurobiological model of fear conditioning have been explained (see Figure 4). This model has been derived from seminal fear conditioning experiments in animals and provides a framework to integrate the empirical findings from the human studies that are part of this thesis, as illustrated in Figure 37. Note that Figure 37 is an extension of Figure 4, which has been described in more detail in chapter 1.2. Some limitations of this "traditional" neurobiological model have already been outlined in chapter 1.2. Addressing several of these shortcomings (see

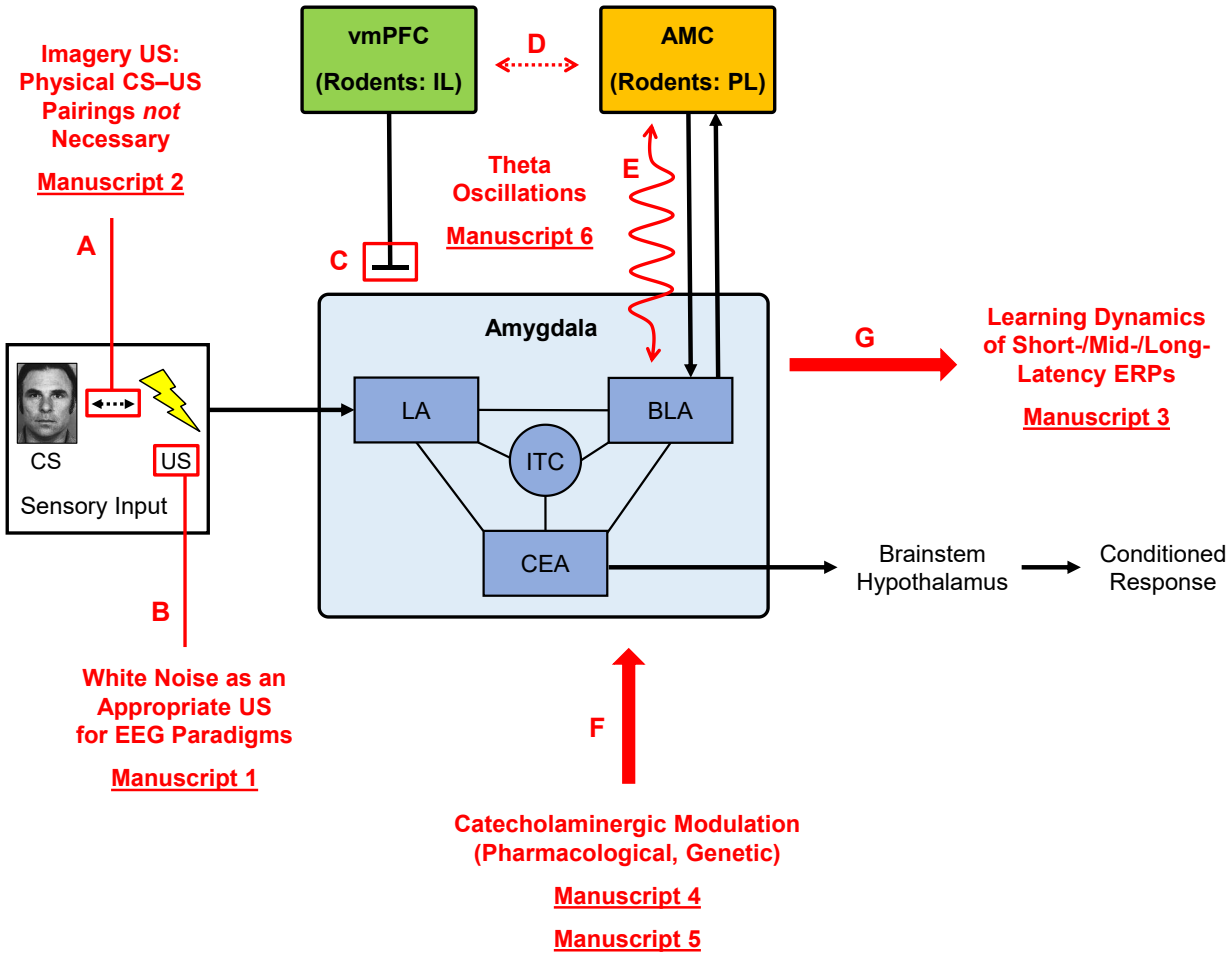


Figure 37. In chapter 1.2, the "traditional" neurobiological model of fear conditioning has been explained (for a more detailed description and an explanation of abbreviations, see the caption of Figure 4). This model provides a framework to integrate the findings of the studies that are part of this thesis.

the manuscripts summarized in chapter 2), the studies of the present thesis have applied a variety of neuroscientific methods (**manuscripts 3, 4, 5, and 6**), clarified important design characteristics (in particular, about the US; **manuscripts 1 and 2**), and suggest a novel sequential-set fear conditioning paradigm (**manuscript 3**).

Manuscripts 1 and 2 addressed open questions about the sensory input (i.e., the US and CS-US pairings), as illustrated in the left part of Figure 37. In **manuscript 1** (see ***arrow B*** in Figure 37), we demonstrated that acoustic white noise bursts seem to be particularly well suited as US for EEG fear conditioning research, given that EEG paradigms typically contain massive numbers of trials (to ensure an adequate signal-to-noise ratio). In **manuscript 2** (see ***arrow A*** in Figure 37), we illustrated that aversive imagery, paired with an initially neutral CS, can cause *de novo* fear conditioning. Remarkably, we obtained similar fear conditioning effects (affective CS ratings, heart period, fear-potentiated startle) as with conventional US types (e.g., unpleasant somatosensory or auditory stimulation). These findings indicate that fear conditioning does not require pairings of CSs with a *physical* aversive US. The neurobiological model displayed in Figure 37 is based on studies with physical USs. fMRI studies suggest that imaging a painful stimulation activates brain networks similar to those involved in pain processing after physical injury (Fairhurst et al., 2012; Jackson et al., 2006; Ogino et al., 2007). Thus, we may speculate that the brain areas illustrated in Figure 37 are also involved in imagery conditioning, but further research applying methods like EEG and fMRI is required to demonstrate formally neural correlates of fear conditioning with an imagined US.

In chapter 1.2, it has been criticized that the "traditional" threat model does not sufficiently include conditioning-induced plasticity in early visual neurons. In **manuscript 3**, we found fear conditioning effects not only on mid- and long-latency brain (EEG) responses, but also modulations of very early (i.e., < 100 ms) brain activity (see ***arrow G*** in Figure 37). Remarkably, we observed fear conditioning effects as early as 33–60 ms after CS onset. Such early EEG effects have often been linked to privileged signal transmission in perceptual areas (Hintze et al., 2014; Mueller & Pizzagalli, 2016; Stolarova et al., 2006; Thigpen et al., 2017). Sparsification of neural representations and enhanced synaptic efficiency have been discussed as important mechanisms for potentiated responses of neurons with ultra-short response latencies (Stegmann et al., 2020; Wieser & Keil, 2020). We were specifically interested in learning dynamics and illustrated how neural responses rise during fear acquisition and vanish during extinction training. The sequential-set fear conditioning paradigm presented in **manuscript 3** is particularly well suited to detect neural activity from brain areas that are prone to habituation. Amygdala activation, which has often been studied with fMRI, shows a rapid habituation curve (see **manuscript 6**). Future studies

should combine our newly developed paradigm with simultaneous EEG-fMRI recordings to better localize brain areas that are related to short-, mid-, and long-latency EEG responses.

In the neurobiological fear conditioning model, AMC-amygdala and vmPFC-amygdala connectivity is assumed to be critical for sustained fear expression and fear extinction, respectively (see chapter 1.2 for further details). However, underlying mechanisms through which these brain areas communicate have remained vague for a long time in human research. In **manuscript 6**, we applied simultaneous EEG-fMRI recordings during fear and extinction recall. We found that effects on frontomedial theta power (EEG) covaried with effects on amygdala activation. Participants with relatively strong amygdala activation to nonextinguished (compared with extinguished) fear stimuli showed relatively strong frontomedial theta power. Theta oscillations, which are assumed to be generated mainly in the AMC (Mitchell et al., 2008; Mueller et al., 2014), seem to play a key role in communication between the amygdala and prefrontal brain areas (in particular, the AMC, see ***arrow E*** in Figure 37). To explain functional coupling between these brain areas during presentation of the CS, two routes may be important: On the one hand, the amygdala may send efferent output to the AMC that modulates the salience of the CS+ and CS- (Gilmartin et al., 2014; Senn et al., 2014), and, in the AMC, these amygdaloid afferents may then be integrated with temporal and contextual information from other brain regions (Fuster, 2001; Gilmartin et al., 2014). On the other hand, excitatory projections from the AMC to the amygdala are thought to be associated with theta synchrony (Bocchio & Capogna, 2014). These projections may convey information about the predictive value of the CS+ and CS- (Courtin et al., 2014; Gilmartin et al., 2014) and, ultimately, regulate amygdala-mediated fear responses (Bocchio & Capogna, 2014; Likhtik et al., 2014; Pitman et al., 2012; Quirk & Mueller, 2008). Furthermore, during extinction learning, the amygdala receives inhibitory input from the vmPFC (Hermann et al., 2016; Milad & Quirk, 2012; Milad, Wright et al., 2007). In the study described in **manuscript 6**, we also observed a negative correlation between frontomedial theta and vmPFC activity, suggesting that there may also be important direct AMC-vmPFC connectivity (see ***arrows C and D*** in Figure 37).

The results from **manuscript 6** may be of great relevance for a better understanding of altered brain processes in patients with anxiety disorders. Exaggerated amygdala activity and deficient prefrontal functioning seem to be important neurobiological mechanisms in pathological fear (Bruhl et al., 2014; Rauch et al., 2006). Moreover, elevated noradrenergic arousal and noradrenaline-mediated projections to the amygdala may lead to hyperconsolidation of aversive memories in the etiology of clinical fear (LaLumiere et al., 2017; McGaugh, 2004, 2015). The studies described in **manuscripts 4 and 5** were conducted with the goal to better understand

catecholaminergic (noradrenaline and dopamine) influences on threat-related brain processes (see ***arrow F*** in Figure 37). **Manuscript 4** assessed the influence of noradrenergic and dopaminergic substances on fear consolidation and extinction learning. In **manuscript 5**, we followed another approach and investigated the influence of catecholaminergic genotypes. Catecholaminergic mechanisms of fear conditioning and extinction are further discussed in chapter 3.2.

3.2 Noradrenergic Hyperconsolidation of Aversive Memories – A Laboratory Model for Pathological Fear?

Noradrenaline is a catecholaminergic neurotransmitter that plays a key role in encoding and consolidating emotional memories (LaLumiere et al., 2017; McGaugh, 2013, 2015). Noradrenergic hyperarousal has been discussed as an important etiological factor (Kalk et al., 2011; O'Donnell et al., 2004; Ronzoni et al., 2016) for fear- and anxiety-related disorders (e.g., PTSD, specific phobias, panic). Specifically, hypervigilance, as a core symptom of pathological fear, is associated with exaggerated activity of the noradrenergic system and abnormally elevated arousal levels (Javanbakht & Poe, 2016; Morris et al., 2020). Yohimbine is a pharmacological substance that experimentally mimics noradrenergic arousal effects (LaLumiere et al., 2017; Schwabe et al., 2013). Prolonged and uncontrollable stress has been linked to heightened vulnerability of the locus coeruleus noradrenaline system (Kapfhammer, 2013; Krystal & Neumeister, 2009), which may further lead to sensitization processes, persistent autonomous hyperarousal, amygdala-mediated overconsolidation of traumatic memories, and impaired extinction learning (Giustino et al., 2020; Hendrickson et al., 2018; Krystal & Neumeister, 2009; McGaugh, 2013, 2015; Roozendaal et al., 2009; Weymar & Hamm, 2013).

In manuscript 4, administration of the noradrenergic substance yohimbine after fear acquisition strengthened fear consolidation processes. Yohimbine led to elevated threat responses one day later, as measured with central (N170 and LPP ERP amplitudes) and peripheral (fear-conditioned bradycardia) physiology. High arousal levels (as experimentally modeled with yohimbine in manuscript 4) after traumatic experiences (i.e., CS-US pairings) have been discussed to potentiate CS-US associations (Kapfhammer, 2013). These consolidation processes, which seem to be mediated through amygdaloid networks, may ultimately contribute to the pathogenesis of clinical fear (Javanbakht & Poe, 2016; Kapfhammer, 2013). Consistent with this idea, subsequent PTSD development has been linked to higher heart rate shortly *after* a traumatic event (Bryant et al., 2000; Shalev et al., 1998). This (neuro-)physiological model for the etiology of pathological fear, which is illustrated in Figure 38, is further supported by our data: Notably, the findings from manuscript 4 suggest that noradrenergic hyperactivity after fear acquisition boosts threat memory consolidation. Thus, our results highlight yohimbine as a striking laboratory model; in future research, the experimental use of yohimbine could allow researchers to uncover neural mechanisms that contribute to the etiology of pathological fear. As outlined in Figure 38, this model may open novel paths for the treatment of fear-related disorders (green boxes). On the one hand, to prevent later transition to PTSD or anxiety disorders, it could be promising to keep arousal levels low (e.g., using relaxation and breathing techniques) in the aftermath of traumatic

experiences (Kapfhammer, 2013; Visser et al., 2015). On the other hand, the pharmacological use of noradrenergic antagonists (drugs with an effect that is opposite to the actions of yohimbine, to *reduce* hyperactivity of noradrenergic neuronal systems) might be another approach to prevent the development of fear-related disorders, such as specific phobias or PTSD (Deng et al., 2020; Soeter & Kindt, 2010, 2011, 2012a, 2012b).

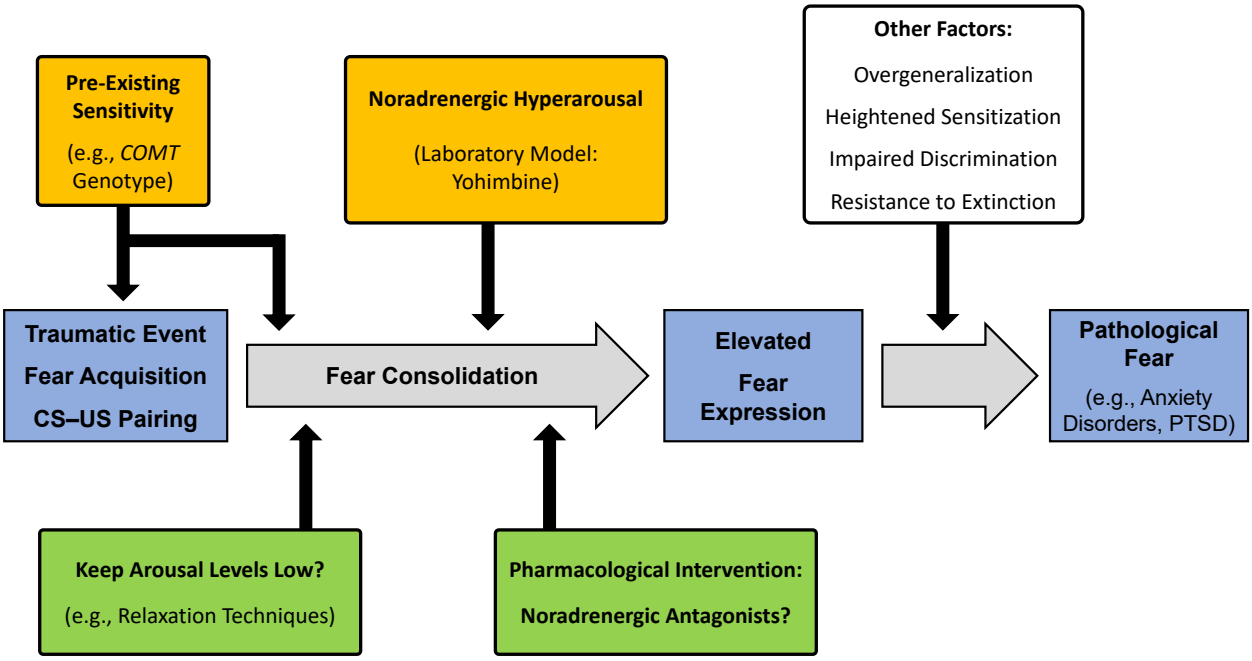


Figure 38. Schematic illustration of catecholaminergic mechanisms during fear conditioning. Noradrenergic hyperarousal (as mimicked by yohimbine) seems to strengthen the consolidation of conditioned fear. These mechanisms are thought to be further modulated by catecholaminergic (e.g., *COMT* Val158Met) polymorphisms (see orange boxes). Potential therapeutic implications might be to keep arousal levels low in the aftermath of a traumatic event, or to reduce noradrenaline activity by noradrenergic antagonists (see green boxes). These suggestions should be clarified in future clinical studies. Other mechanisms that are relevant for the development of pathological fear are overgeneralization to non-associated cues, heightened sensitization, impaired discrimination between threat and non-threat cues, and resistance to extinction (see white box).

In manuscript 4, we showed that the noradrenergic substance yohimbine strengthened fear consolidation. We did not find effects of the dopaminergic substance sulpiride, which was also assessed in this study. Thus, it is tempting to speculate that noradrenaline could be more important for fear consolidation mechanisms than dopamine. However, our experimental design does not allow us to exclude dopaminergic effects totally. In fact, manuscript 5 of this thesis investigated

the effects of dopamine-related *COMT* Val158Met genotypes and suggests dopaminergic effects on fear and extinction recall. Figure 38 illustrates that catecholaminergic polymorphisms (e.g., *COMT* Val158Met) may modulate the preexisting sensitivity to acquire (and extinguish) fear. In the study reported in manuscript 5, Val/Val carriers (compared with Val/Met and Met/Met) showed elevated fear recall for nonextinguished threat stimuli 24 hours after acquisition, as evident in fear-conditioned bradycardia and LPP amplitudes. *COMT* mRNA is highly expressed in the prefrontal cortex (Matsumoto et al., 2003). In the literature (Bilder et al., 2004; Meyer-Lindenberg & Weinberger, 2006; Yavich et al., 2007), Val/Val homozygosity has mostly been linked to relatively lower prefrontal dopamine levels (see chapter 1.6, in particular, Figure 14). However, as outlined in chapter 1.6, the COMT enzyme is *not* specific for dopamine breakdown: It is also involved in noradrenaline degradation (see Figure 11). Indeed, some authors have emphasized that the effects of *COMT* genotype cannot be tied cleanly and exclusively to dopaminergic mechanisms, but may also be associated with effects on noradrenaline activity (Ehlers & Todd, 2019; Javanbakht & Poe, 2016). Noradrenergic and dopaminergic mechanisms on fear conditioning and extinction most likely interact. Individual differences in fear learning seem to be related to the interplay of several (probably hundreds) genetic polymorphisms (Ehlers & Todd, 2019). In manuscript 5, we found the most adaptive response pattern (i.e., high fear *and* extinction recall) for Val/Val carriers. Further research is required to elucidate whether and how the *COMT* Val158Met polymorphism is associated with better fear consolidation during high states of noradrenergic arousal (as illustrated in Figure 38). The interplay of certain catecholamine-related genotypes (as a preexisting sensitivity) and noradrenergic hyperarousal after traumatic events may ultimately terminate in the development of clinical fear (e.g., anxiety disorders and PTSD).

For the present thesis, we performed *two* studies to investigate pharmacological actions (manuscript 4) and genetic variations (manuscript 5) that are related to catecholaminergic mechanisms. To better disentangle noradrenergic and dopaminergic processes, future research should *combine* pharmacological interventions (e.g., yohimbine and sulpiride) *and* genotyping (noradrenergic and dopaminergic polymorphisms; e.g., *COMT* Val158Met polymorphism) in *one* study with a larger dataset. This approach would allow testing interactions between pharmacological and genetic factors. Ultimately, this research line could help clarify the relative importance of noradrenaline and dopamine in threat processing.

Manuscript 5 addressed genotype effects on fear-conditioned bradycardia, which may contribute to individual differences in anticipatory threat expression. Animal and human research suggest that these genetic effects may be mediated through extended brain networks including prefrontal and amygdaloid regions (Schipper et al., 2019). Similarly, threat-induced release of

noradrenaline and dopamine is thought to modulate brain activity in the amygdala and prefrontal cortex (Burgos-Robles et al., 2009; Giustino & Maren, 2018; Pezze & Feldon, 2004). In manuscripts 4 and 5, EEG was used to measure neural responses, but the spatial precision of this method is limited (Hajcak et al., 2019). Future studies should combine pharmacological and genetic methods with simultaneous EEG-fMRI recordings to better elucidate relevant brain regions that mediate catecholaminergic mechanisms. The simultaneous EEG-fMRI technique has been successfully applied in manuscript 6 of this thesis.

According to the model (see Figure 15 in chapter 1.7) introduced by Ressler and colleagues (Bowers & Ressler, 2015; Maddox et al., 2019; Parsons & Ressler, 2013; Ross et al., 2017), pathological fear is characterized by overgeneralization to non-associated cues, heightened sensitization, impaired discrimination between threat and non-threat cues, and resistance to extinction. While we found noradrenergic effects on central (N170, LPP) and peripheral (heart rate) correlates of fear recall, future studies should elucidate how yohimbine influences neural signatures of fear generalization, sensitization, discrimination, and extinction learning (see white box in Figure 38). To reduce variance related to fluctuations of gonadal hormones, only male participants were included in the studies on catecholaminergic mechanisms (manuscripts 4 and 5). Sex differences and personality traits (which are discussed in chapter 3.3) may further modulate noradrenergic effects on conditioned and extinguished fear (Lonsdorf & Merz, 2017; Merz et al., 2018; Schwabe et al., 2013).

3.3 Physiological Correlates of Fear Conditioning – A Matter of Sex and Personality?

Future studies should investigate whether the findings of this thesis are modulated by personality differences or sex hormone status. The consideration of individual differences would allow researchers to improve the signal-to-noise ratio of (neuro-)physiological signals by further reducing unexplained variance, which is otherwise regarded as "noise" in statistical analyses (Lonsdorf & Merz, 2017). There is robust evidence that the strength of conditioned responses during fear acquisition, fear extinction, and fear/extinction recall differs between men and women, and threat responses are particularly modulated by the menstrual cycle phase (for a recent review, see Merz et al., 2018). To control for potential interactions between catecholaminergic mechanisms and sex hormone status, only males were tested in the studies described in manuscripts 4 and 5. The restriction to male samples is appropriate to ensure internal validity, but this approach can be highly problematic in terms of external validity – given that women are at twofold greater risk of developing pathological fear (Christiansen & Berke, 2020; Ramikie & Ressler, 2018; Seligowski, Harnett et al., 2020; Seligowski, Hurly et al., 2020). In fact, sex differences in the locus coeruleus noradrenaline system may be responsible for increased noradrenaline synthesis and elevated arousal levels in females (Bangasser et al., 2016), a factor that may further explain the higher prevalence of clinical fear among women (see also chapter 3.2). Future research is necessary to study whether our findings on catecholaminergic mechanisms (i.e., manuscripts 4 and 5) generalize to females and whether effects are modulated by sex hormones, menstrual cycle, or oral contraceptives.

Low estrogen levels have been associated with stronger fear recall (Barha et al., 2010; Kobayashi et al., 2020; Merz et al., 2018) and deficient extinction recall (Graham & Milad, 2013; Merz et al., 2018; Zeidan et al., 2011). Rodent research has demonstrated that impaired extinction recall in females was associated with enhanced PL theta oscillations (Fenton et al., 2014) and reduced IL gamma oscillations (Fenton et al., 2016). In the study of manuscript 6, in which we assessed oscillatory theta activity during fear and extinction recall, we aimed to reduce variance related to fluctuations of gonadal hormones. Thus, free-cycling women were excluded. In addition to males, we recruited only female participants who took oral contraceptives on a regular basis. All females were tested during their pill intake phase. As part of a recent collaborative study with the University of Osnabrueck (Bierwirth, Sperl, Antov, & Stockhorst, 2021), we specifically explored the influence of estrogen status on fear and extinction recall. To this end, we recruited men, women using oral contraceptives (i.e., low estradiol [E2] and low progesterone [P4]), and free-cycling women during mid-cycle (i.e., high E2 and low P4). We applied the same two-day

fear conditioning and extinction paradigm that was used in manuscripts 1, 4, 5, and 6 of the present thesis. Remarkably, in this collaborative study, we found reduced fear expression on day 2 for mid-cycle women (high E2) compared with those women taking oral contraceptives and men (both low E2). Sex differences were indicated by reduced SCRs and attenuated AMC-localized theta oscillations (see Figure 39).

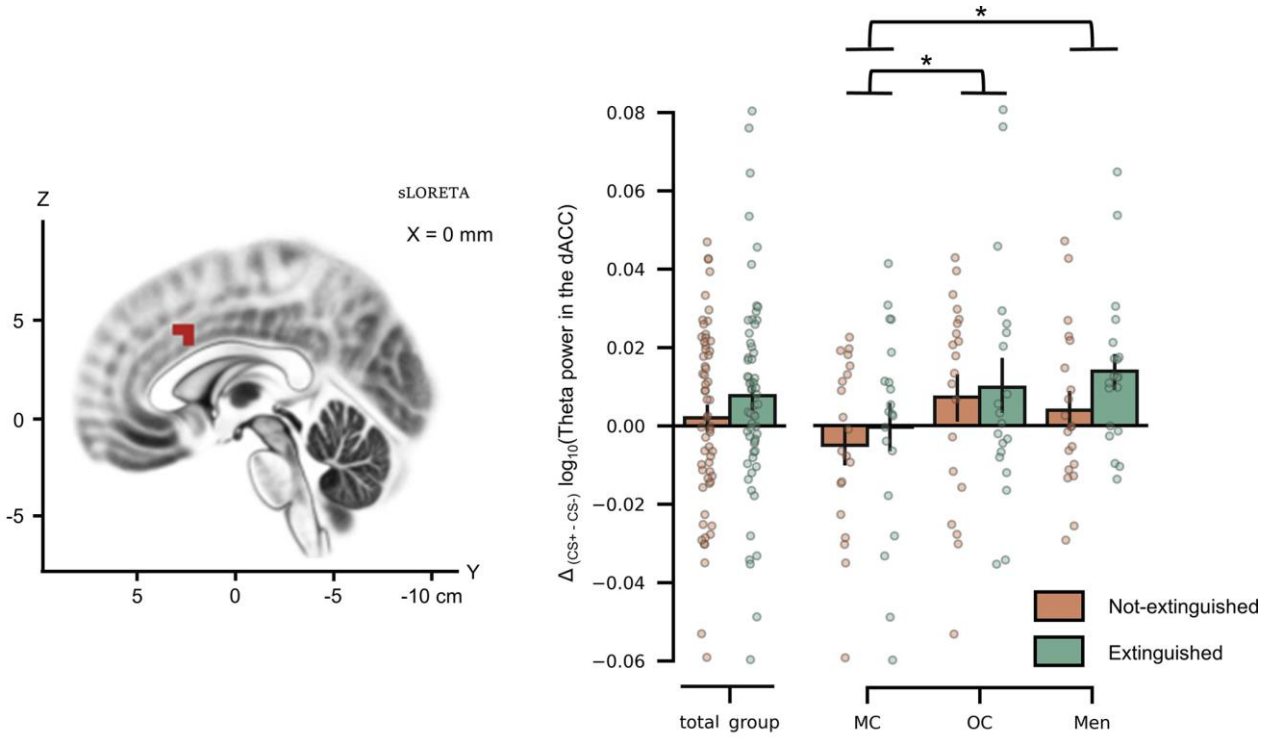


Figure 39. In a recent collaborative study with the University of Osnabrueck (Bierwirth, Sperl, Antov, & Stockhorst, 2021), we have found that estradiol-status modulates AMC/dACC source-localized (standardized low resolution brain electromagnetic tomography; sLORETA) theta power. The left panel illustrates the AMC/dACC region of interest (ROI), Montreal Neurological Institute (MNI) coordinates $X = 0$ mm, $Y = 18$ mm, $Z = 34$ mm. Bars show mean differential (CS+ minus CS-) current density theta power (4–8 Hz) in the AMC/dACC for nonextinguished (orange) and extinguished (green) CSs. Differential current density theta power is shown (from left to right) for the total group, MC-women, OC-women (OC = oral contraceptives), and men. Error bars show ± standard error of the mean (*SEM*). Asterisks indicate significant group differences from post hoc *t*-tests for independent samples. * $p \leq .05$. Figure republished from Bierwirth, Sperl, Antov, & Stockhorst (2021).

In the above-mentioned collaborative study (Bierwirth, Sperl, Antov, & Stockhorst, 2021), in manuscript 6 of this thesis (Sperl et al., 2019), and in the study by Mueller et al. (2014), oscillatory theta activity was modulated by conditioned responses 24 hours after fear conditioning and extinction. In all of these studies, we did not observe elevated theta power for CS+ compared with CS- during the *acquisition* stage on the first day. The absence of theta effects during fear

acquisition (which was formally tested in two datasets: Mueller et al., 2014; Bierwirth, Sperl, Antov, & Stockhorst, 2021) suggests the interpretation that elevated theta power is rather related to fear *recall* than fear *acquisition*. However, as part of another ongoing collaborative project (Wroblewski, Sperl, Mueller, Mueller, & Straube, in preparation), we have reanalyzed the EEG data of manuscript 3. While manuscript 3 focused on ERP data (see chapter 2.3), we have also assessed oscillatory theta power during this reanalysis. In this dataset, we also found elevated theta power for CS+ compared with CS- during the fear *acquisition* stage on day 1. Theta activity during acquisition training has further been source-localized to brain regions including the AMC (see Figure 40). Future research is required to better clarify under which conditions theta modulations can also be observed during fear acquisition. This, in turn, would allow researchers to narrow and to specify the interpretation with regard to functional meanings of theta oscillations in the context of fear expression.

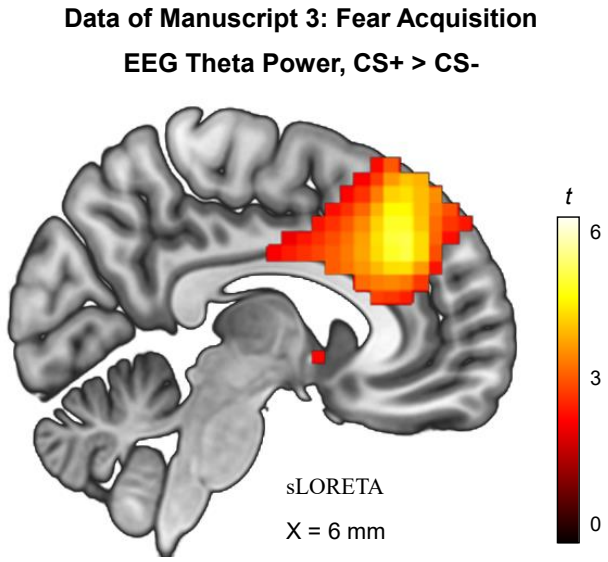


Figure 40. As part of a current collaborative study (Wroblewski, Sperl, Mueller, Mueller, & Straube, in preparation), we have reanalyzed the EEG data of manuscript 3 with regard to oscillatory theta activity. During day 1 acquisition, we found elevated theta power for CS+ compared with CS- at frontomedial EEG channels, which has been further source-localized (using standardized low resolution brain electromagnetic tomography; sLORETA) to brain regions including the AMC. For illustrative purposes, the intensity threshold was set to $p \leq .05$.

Besides the influence of gonadal hormones (e.g., estradiol status), several studies have explored associations between trait anxiety/neuroticism and fear conditioning (e.g., Gazendam et al., 2013; Haaker et al., 2015; Kindt & Soeter, 2014; Martínez et al., 2012; Pineles et al., 2009; Sehlmeyer et al., 2011; Sjouwerman et al., 2020; Sperl et al., 2016; Staples-Bradley et al., 2018; Torrents-

Rodas et al., 2013; Vriends et al., 2011; Wiggert et al., 2017). Previous studies examining the influence of trait anxiety and neuroticism on conditioned responses have often been inadequately powered (i.e., too small sample sizes) and have produced highly inconsistent findings: While some studies have revealed positive associations between trait anxiety and fear conditioning (which could indicate an enhanced vulnerability to anxiety disorders in high-anxious individuals), others have reported negative (which may be interpreted as elevated fear generalization to the CS- non-threat cue and deficient safety learning) or no correlations (for a recent overview: Lonsdorf & Merz, 2017). In manuscript 1, we performed additional analyses on state and trait anxiety. Arousal ratings revealed slower conditioning for individuals with high state anxiety, but this correlation pattern was restricted to the electric shock group. Furthermore, in the white noise burst group, SCRs indicated stronger fear conditioning for participants with low trait anxiety and neuroticism scores. Given the discrepant findings in the literature (i.e., positive correlation, negative correlation, no correlation; see above), trait anxiety and neuroticism may be invalid predictors of conditioned fear responses in laboratory settings. In manuscript 5 of this thesis, we also performed analyses on personality measures, in addition to the research questions regarding the *COMT* Val158Met polymorphism that have been reported in chapter 2.5. In this manuscript, we argue that trait *fearfulness* might be a more suitable predictor for fear conditioning. The differentiation between trait anxiety and fearfulness is related to the distinction between state anxiety and fear, which has already been discussed briefly in chapter 1.1 of this thesis. High trait anxiety and neuroticism are thought to predispose individuals to *proactive* behavior in rather *ambiguous* situations, with the goal to avoid more *distant* threats (Blanchard et al., 2001; McNaughton, 2011; Perkins & Corr, 2006). By contrast, high trait fearfulness describes the disposition for *reactive* behavior (e.g., fight, flight, freeze responses) in *unambiguously* harmful and dangerous situations, aiming to protect oneself from *imminent* threat (Blanchard et al., 2001; McNaughton, 2011; Perkins & Corr, 2006; Sylvers et al., 2011). Fearfulness could be a better predictor for conditioned fear responses, given that CSs are cues that *reliably co-terminate* with the occurrence of a *clearly* aversive US. In manuscript 5, greater fearfulness (but not trait anxiety and neuroticism) was associated with larger conditioned fear responses, as measured with subjective CS ratings and heart period changes (see Figure 41). There was also a positive dose effect of the Val allele on fearfulness scores, linking personality findings to the results described in chapter 2.5. Together, these results suggest that trait fearfulness might be a better predictor for conditioned fear responses than trait anxiety or neuroticism. Note that trait fearfulness and trait anxiety were not correlated in manuscript 5.

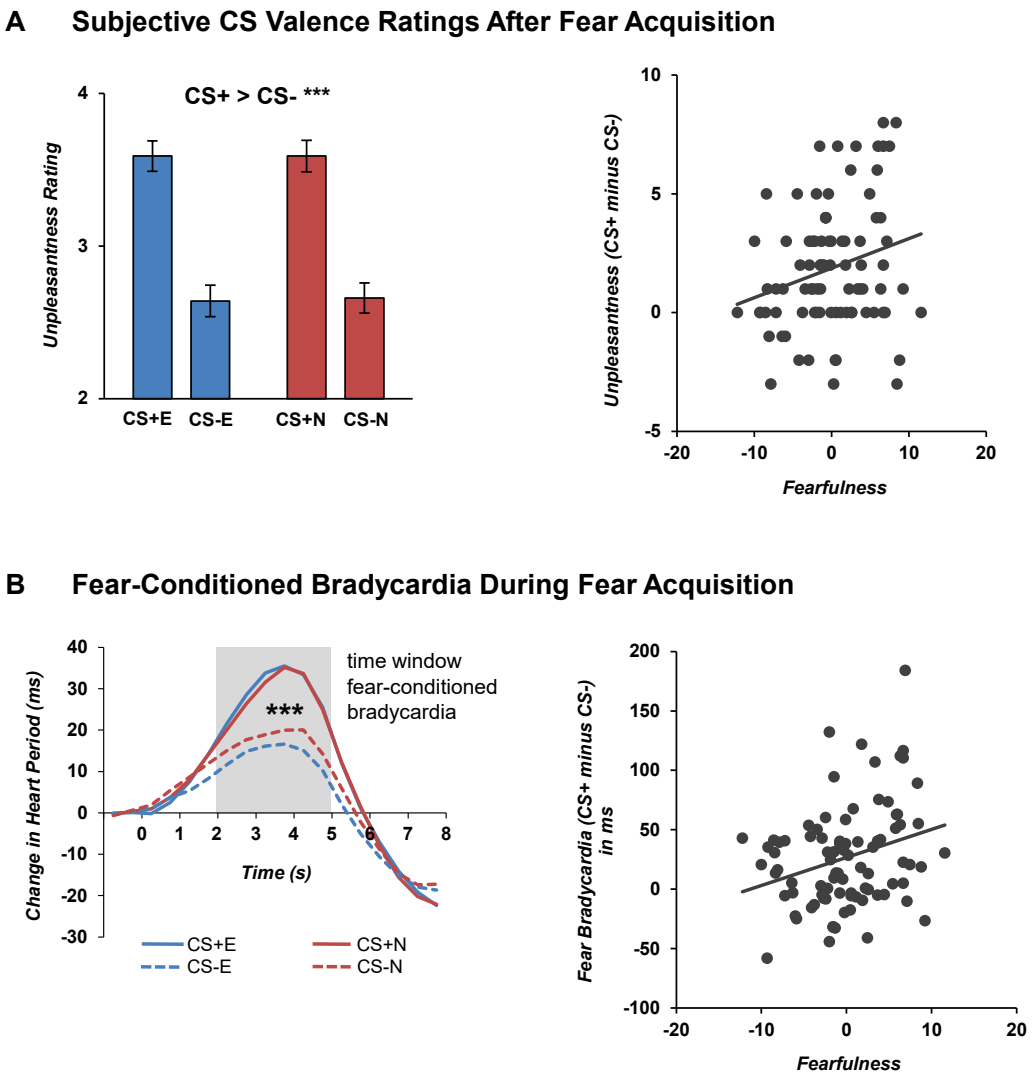


Figure 41. Subjective CS ratings and heart period data that are illustrated in this figure are taken from manuscript 5 of this thesis (see chapter 2.5). Conditioning effects on (A) negative valence ratings (assessed at the end of fear acquisition) and (B) fear-conditioned bradycardia (assessed during fear acquisition) are shown in the left panels. Their relationship with trait fearfulness is shown in the right panels. Error bars indicate *SEM*s, based on within-subject variance. *** $p \leq .001$ for main effect of *Contingency* (CS+ versus CS-). CS+N and CS-N: nonextinguished stimuli; CS+E and CS-E: extinguished stimuli. Higher fearfulness predicted (A) larger differential (CS+ minus CS-) unpleasantness ratings and (B) larger fear-conditioned bradycardia (i.e., relative heart rate deceleration for CS+ compared with CS-). Figure republished from Panitz, Sperl et al. (2018).

In manuscript 6 of this thesis, we found between-subjects correlations between EEG and fMRI measures (theta-amygdala and theta-vmPFC activity), suggesting that the strength of neural responses to fear-conditioned stimuli varies among individuals. However, it remains unclear whether this correlation pattern is related to individual differences like fearfulness and estradiol status. In many neuroscientific fear conditioning studies (including most studies of the present

thesis), the sample sizes are too small to investigate sex and personality effects appropriately with sufficient power. To uncover these important moderator variables, future EEG and fMRI studies should collect larger samples (e.g., through cross-lab collaborations). Besides genetics (see manuscript 5 of this thesis), these moderator variables may include personality measures (e.g., fearfulness), gonadal hormones (e.g., estradiol), stress hormones, life history, brain morphology, and developmental stage (Lonsdorf & Merz, 2017). In addition to biological sex, future studies should also assess factors that are related to sociocultural gender (e.g., "masculinity", "masculine ideals," and "masculine gender role stress" in the context of anxiety and PTSD symptoms; Christiansen & Berke, 2020). On the one hand, the statistical consideration of these (and further) variables turns "noise" into a meaningful tune, resulting in greater statistical power and more robust findings on neural fear conditioning correlates. On the other hand, this strategy may open promising paths for translational science – given that fearfulness, as an example, might be particularly important for etiological models of anxiety disorders, such as phobias and panic disorder (Corr, 2009; Kampman et al., 2017; Lang et al., 2000; McNaughton & Corr, 2004; Sylvers et al., 2011).

3.4 Future Research Directions: From Fear Conditioning to Neuroscience and Back?

"Fear" is part of an alarm system that lets individuals know that they might be in danger. Relevant concepts have been explained in more detail in chapter 1. Fear can be critical for survival – because it allows individuals to take steps to protect themselves from potential harm. As highlighted in the manuscripts of the present thesis, fear is accompanied by changes in central (e.g., measured with EEG and fMRI) and peripheral (e.g., as indicated by SCRs, heart period changes, and fear-potentiated startle responses) physiology. These responses prepare the body to fight or to flee unsafe situations, or help to avoid such threatening situations in the future. The subjective state of "fear" is associated with physiological and behavioral changes. These responses can also be *learned* through Pavlovian conditioning ("fear conditioning").

The present thesis demonstrates that EEG is a promising tool for neuroscientific fear conditioning research – provided that methodological challenges are addressed adequately. In addition to the selection of a suitable US (see manuscripts 1 and 2 of this thesis), experimental paradigms need to be sensitive to habituation-prone neural responses (see manuscripts 3 and 6). They should be able to detect temporal changes over time (see manuscript 3). Furthermore, EEG analyses should not only focus on ERP components (see manuscripts 3, 4, and 5 of this thesis), but also capture oscillatory brain activity (see manuscript 6). Finally, the combination of EEG and other neuroscientific methods (e.g., fMRI, see manuscript 6 of this thesis) allows researchers to bridge the gap between animal (theta oscillations, amygdala activity), human EEG (theta oscillations), and human fMRI (amygdala activity) findings.

The results of the present thesis add further evidence to *methodological* and *translational* directions in current neuroscientific fear research. Figure 42 illustrates which manuscripts address more methodological or more translational research questions. Note that this visualization is a simplification, with the goal to better highlight the key findings of each study. Although in each study we focused on one specific aspect, most of the manuscripts in this thesis actually address *both* methodological *and* translational issues. Manuscripts 1 and 2 investigated mainly methodological research questions regarding the US in fear conditioning studies. Besides its methodological value, manuscript 2 is also highly important in terms of a translational understanding of fear conditioning. In the two studies described in manuscript 2, we demonstrated that *de novo* fear conditioning is possible with *imagined* USs, without any physical US presentation. Given that many patients with anxiety disorders cannot recall the experience of traumatic events, our findings bridge between clinical models and basic fear conditioning

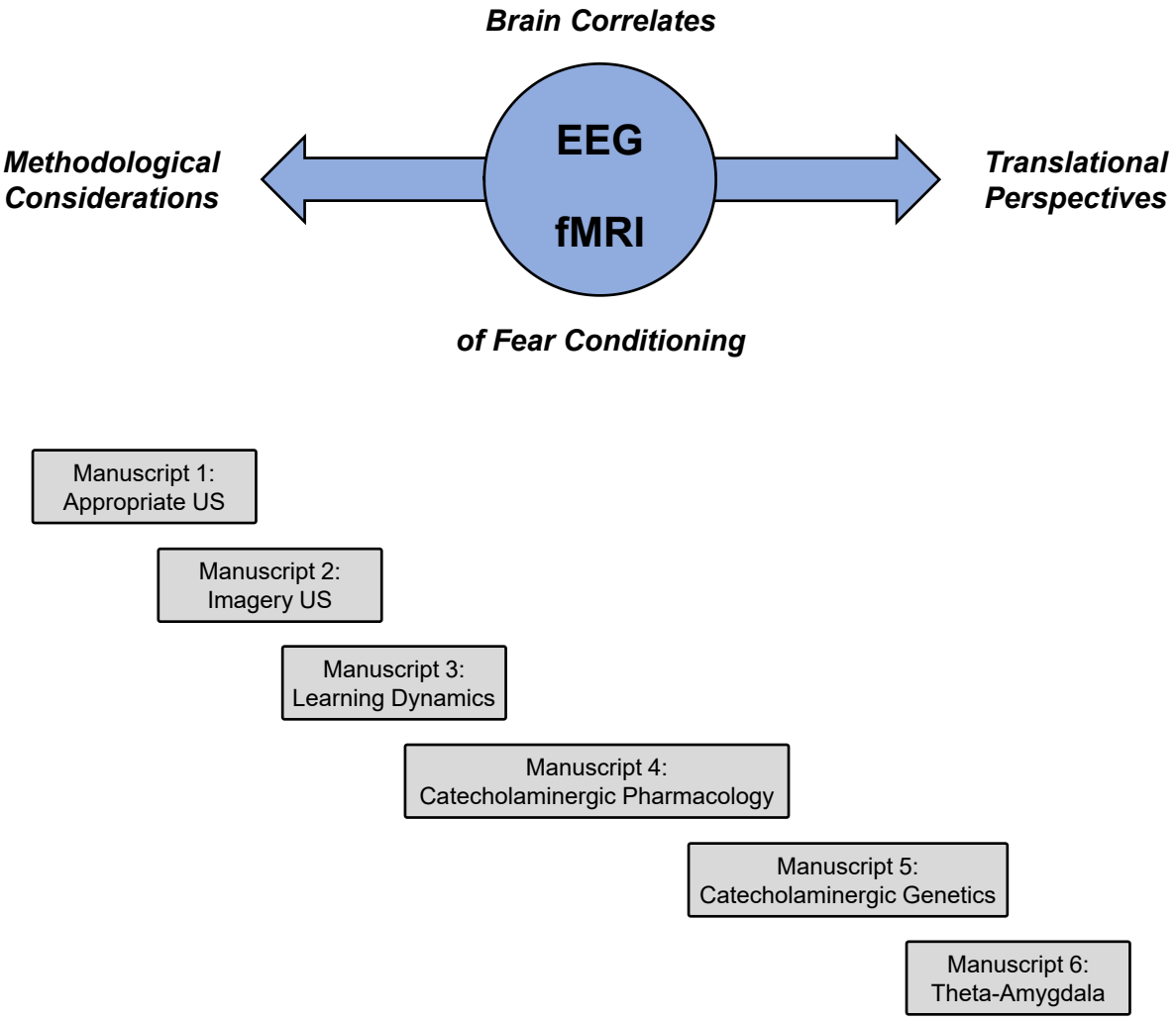


Figure 42. The present thesis comprises six manuscripts, as summarized in chapter 2. In these manuscripts, EEG and fMRI methods have been used to elucidate brain mechanisms involved in fear conditioning, with a special focus on methodological considerations and translational perspectives.

paradigms in animals and humans, which often include *physical* CS-US pairings. Similarly, manuscript 3 adds both translational and methodological knowledge. On the one hand, this study furthers our understanding about the speed of cortical threat processing, which is, to a large extent, based on animal research. On the other hand, we present a new paradigm for EEG research that allowed us to uncover learning dynamics of electrophysiological brain signals. While manuscript 1 clarifies how to avoid habituation to the US, manuscript 3 suggests a paradigm that circumvents habituation to the CSs; this ability is a prerequisite to uncover the learning curve of electrocortical threat responses with a sufficiently high signal-to-noise ratio. Manuscript 4 highlights how yohimbine administration facilitates fear consolidation, as measured with peripheral physiology and EEG. This study offers exciting translational perspectives, demonstrating that yohimbine is a

powerful experimental tool to elucidate noradrenergic mechanisms in fear processing. Furthermore, in the long term, the locus coeruleus noradrenaline system may be a promising target for interventions aiming to prevent or to treat pathological fear. In a similar way, manuscript 5 demonstrates how the catecholamine-related *COMT* Val158Met polymorphism modulates fear and extinction recall. In light of stratified and precision mental health care, these findings may be highly relevant for the design of individually tailored interventions for patients with fear-related disorders. Finally, we applied advanced neuroscientific methods (simultaneous EEG-fMRI recordings) in manuscript 6 to bridge the gap between animal findings (prefrontal theta oscillations) and human fMRI research (amygdala activity). Similarly to the previous studies, manuscript 6 combines promising methodological (EEG-fMRI integration) and translational (transferring animal findings on oscillatory brain activity to humans) directions.

Besides the conclusions discussed above and in the previous sections, our findings are also highly relevant for theoretical conceptualizations of fear conditioning. The neurobiological fear conditioning model (see Figure 4 in chapter 1.2, and Figure 37 in chapter 3.1) assumes that projections from the central nucleus of the amygdala (CEA) regulate peripheral physiological responses to threat. In the studies presented in the current thesis, we applied several peripheral physiological methods (skin conductance, heart period, and fear-potentiated startle). In addition to the research questions summarized above, these peripheral physiological findings are very well suited to explain and correct a common misunderstanding that often leads to incorrect definitions of fear conditioning. A contemporary American textbook of applied clinical psychology (Barlow et al., p. 24), for example, describes classical conditioning as follows:

> "In his classic study examining why dogs salivate before the presentation of food, physiologist Ivan Petrovich Pavlov (1849–1936) of St. Petersburg, Russia, initiated the study of classical conditioning, a type of learning in which a neutral stimulus is paired with a **response until it elicits <u>that</u> response**."
>
> (Barlow, Durand, & Hofmann, 2018, *Abnormal Psychology – An Integrative Approach*, p. 24; bold and underlined emphasis added by M. F. J. Sperl.)

This definition ("*<u>that</u>* response") implies that conditioned responses and unconditioned responses are *identical*. It has already been pointed out in an early commentary by Rescorla (1988, pp. 151 and 158) that this assumption is *not* justified:

> "Traditional descriptions of conditioning as the acquired ability of one stimulus to **evoke the original response** to another because of their pairing are shown to be **<u>inadequate</u>**. […] The implication is that describing Pavlovian conditioning as the endowing of a **CS with the ability to evoke the same response as the US** is a **<u>wholly inadequate characterization</u>**. Pavlovian conditioning is **<u>not</u> the shifting of a response from one stimulus to another**. Instead, conditioning involves the **learning of relations** among events that are complexly represented, a learning that can be exhibited in various ways. We are badly in need of an adequate theory of

> performance in Pavlovian conditioning, but the classical notion of a **new stimulus taking on the ability to evoke an old response clearly will <u>not</u> do**."
>
> (Rescorla, 1988, Pavlovian Conditioning: It's Not What You Think It Is. *American Psychologist*, *43*, 151–160; bold and underlined emphasis added by M. F. J. Sperl.)

This view has been emphasized in a recent opinion paper by Fanselow (2018). Neuroscience is not only a useful tool to elucidate physiological correlates of fear conditioning. In fact, Rescorla (1988, pp. 151 and 159) recognized relatively early that neuroscientific findings can also be extremely helpful to advance the conceptual understanding of fear conditioning mechanisms:

> "Pavlovian conditioning is one of the oldest and most systematically studied phenomena in psychology. Outside of psychology, it is one of our best known findings. But at the same time, within psychology it is badly misunderstood and misrepresented. […] **Neuroscientists have decided, quite rightly I believe, that Pavlovian conditioning provides one of the best-worked-out learning situations for them to analyze.** It has a well-developed data base that can be characterized quite successfully by available theories. The hopeful sign is that, increasingly, neuroscientists are familiarizing themselves with the contemporary state of Pavlovian conditioning and are attempting to account for a host of new results, such as sensitivity to information, inhibitory learning, and so forth. **Indeed, many neuroscientists are better acquainted with the modern state of Pavlovian conditioning than are psychologists at large. It is partly through that acquaintance that genuine progress is being made in the biological analysis of learning.**"
>
> (Rescorla, 1988, Pavlovian Conditioning: It's Not What You Think It Is. *American Psychologist*, *43*, 151–160; bold emphasis added by M. F. J. Sperl.)

Although Rescorla had already pointed out these important conceptualizations in 1988, they are still often ignored in current research and contemporary textbooks (see example above). Indeed, neuroscientific findings from the studies of the present thesis support Rescorla's understanding that conditioned and unconditioned responses are *not* identical. In manuscript 2, we studied fear conditioning with imagined USs. For skin conductance, we found that the imagined US evoked elevated SCRs, but the CS+ did not. For heart period, we observed cardiac *ac*celeration for the US, but cardiac *de*celeration for the aversive CS+ (compared with the neutral CS+). Similar findings were obtained in manuscript 3: The electrotactile US was associated with heart rate *ac*celeration, but the CS+ (compared with the CS-) was followed by relative *de*celeration (see Figure 9 in chapter 1.5). Furthermore, the EEG analyses in manuscript 3 demonstrated that the US compared with CS+/- also evoked different electrocortical (ERP) responses (see Figure 27 in chapter 2.3). Thus, conditioned and unconditioned responses differ not only in peripheral physiology, but also in neurophysiological signatures.

At first glance, diverging autonomic responses for CSs versus USs, which even go in the opposite direction (cardiac deceleration versus acceleration), may seem paradoxical. However, this response pattern gives critical insight into the functional meaning of physiological changes during

fear conditioning, which is related to different stages of threat proximity (Davis & Lang, 2003; Löw et al., 2015; Mobbs et al., 2020; Obrist, 1976). Cardiac deceleration during the anticipation of threat (i.e., during CS+ presentations) can be linked to heightened vigilance, allocation of attentional resources, and facilitated sensory intake, preparing the organism for later action responses (Lang & Bradley, 2010; Roelofs, 2017). Conversely, cardiac acceleration at a time when threat is most imminent (i.e., during US presentations) is associated with systemic activation and active fight-or-flight behavior (Davis & Lang, 2003; Lang & Bradley, 2013; Löw et al., 2015). This example illustrates how psychophysiological research can help to refine conceptualizations and to elaborate mechanisms that are involved in fear conditioning.

As already emphasized by Rescorla (1988) and further supported by the empirical data of this thesis, the CS does *not* necessarily evoke the same response as the US. A more promising and more accurate way is to define fear conditioning with regard to learning about *relations* between stimuli or events, as it has been done in chapter 1.1 ("the CS elicits a given conditioned response only *conditionally* after it has been paired with the US, which generates the unconditioned response *unconditionally*"; see page 19). A similar approach would be to describe fear conditioning in terms of changing CS-dependent *expectations* about the US occurrence (Rief et al., 2015). According to this view, a discrepancy between US *expectation* and the actual US occurrence would be crucial for successful fear conditioning and extinction. In other words, *breaking expectations* about stimulus occurrence and intensity seems to represent the key mechanism of associative learning. The current thesis has been prepared as part of the Research Training Group (RTG) 2271 "Breaking Expectations: Maintenance versus Change of Expectations in the Context of Expectation Violations," which is funded by the German Research Foundation (Deutsche Forschungsgemeinschaft; DFG) and located at the University of Marburg.

Expectations can be defined as future-oriented conditional beliefs regarding the probability of the incidence or non-incidence of events, states, or experiences (Hoorens, 2012; Kube et al., 2020; Laferton et al., 2017; Olson et al., 1996; Pinquart et al., 2021; Rief & Joormann, 2019). Expectations guide behavior, and learning can be understood as adjustments of expectations (Gollwitzer et al., 2017; Rief et al., 2015; Rief & Glombiewski, 2016). In particular, conditioning procedures are considered to be major mechanisms in the development of expectations (Klinger et al., 2007; Pinquart et al., 2021; Rief et al., 2015). Dysfunctional expectations are core features in the etiology and maintenance of mental disorders, including anxiety disorders (Boehme et al., 2014; Gu et al., 2020; Kube et al., 2020; Qi et al., 2017; Rief et al., 2015). The violation of dysfunctional expectations (e.g., through exposure therapy) is a vital goal of psychotherapy (Craske et al., 2018; Rief et al., 2015; Rief & Glombiewski, 2016). Rief et al. (2015) have

introduced a model (the so-called "violated expectation model") that provides a framework to describe factors that are related to the persistence and change of expectations. This model has been revised by our group (Panitz, Endres, Buchholz, Khosrowtaj, *Sperl*, Mueller, Schubö, Schütz, Teige-Mocigemba, & Pinquart, submitted to *Frontiers in Psychology*), and extended with regard to clinical applications (Rief, Khosrowtaj, Körfer, Panitz, Sarter, Schäfer, Schwarting, *Sperl*, & Teige-Mocigemba, in preparation). This model (Gollwitzer et al., 2017; Panitz, …, Sperl et al., submitted; Rief et al., 2015; Rief & Glombiewski, 2016; Rief, …, Sperl, & Teige-Mocigemba, in preparation) specifies cognitive responses to expectation violations in a specific situation (accommodation, immunization) and anticipatory behavioral responses that aim to change or decrease the probability of expectation violation (assimilation, experimentation). The conceptualization of fear conditioning in terms of this expectation-focused framework may open up new and innovative directions for neuroscientific investigations of threat. These research directions may further extend the findings of the present thesis.

According to this expectation-oriented theory, *accommodation* describes the mechanism associated with an adjustment of expectations after an expectation violation (Gollwitzer et al., 2017; Panitz, …, Sperl et al., submitted; Rief et al., 2015; Rief & Glombiewski, 2016; Rief, …, Sperl, & Teige-Mocigemba, in preparation). Thus, accommodation can be considered a goal of exposure therapy in the treatment for anxiety disorders, given that this technique aims to promote extinction learning by experiencing expectation-disconfirming situations (Craske et al., 2018; Rief et al., 2015; Rief & Glombiewski, 2016; Rief, …, Sperl, & Teige-Mocigemba, in preparation). Manuscripts 3 and 6 of this thesis elucidate neural mechanisms (ERP amplitudes, oscillatory brain activity, hemodynamic responses) that go along with extinction-induced changes of expectations (i.e., accommodation). In manuscript 4, however, we were unable to strengthen extinction learning by administering noradrenergic or dopaminergic substances. Future research should investigate whether understanding fear extinction in terms of *expectation violation* (Craske et al., 2018; Lipp et al., 2020) may be a promising approach to boost extinction learning and to facilitate the efficacy of exposure therapy. In an ongoing collaborative project, we are assessing the effect of exposure instructions (i.e., instructions that focus either on habituation processes or expectation violations) on extinction learning.[1] The goal of our current research project (Sperl, Körfer et al., in preparation) is to probe whether fear extinction can be facilitated (i.e., faster and stronger decline of conditioned fear responses) when participants are explicitly instructed to *test their expectations*

[1] Together with Dr. Karoline Körfer (University of Marburg, Department of Clinical Psychology), I have successfully applied for an RTG 2271 "Treasure Box" funding (German Research Foundation, DFG) for this joint research project, which is focused on the interplay of exposure instructions, fear extinction, and clinical applications.

(Kleine et al., 2017; Körfer et al., 2020; Schemer et al., 2020) about the CS-US contingency during the extinction session. In future studies, this approach should be combined with EEG and fMRI (see manuscripts 3, 4, 5, and 6 of this thesis) to test whether facilitated extinction learning is also associated with changes in neural responding. Ultimately, our results may help to identify new targets for therapeutic interventions and improve the efficacy of exposure therapy in patients with pathological fear conditions.

Immunization refers to cognitive mechanisms aiming to minimize the impact of expectation-disconfirming experiences (Gollwitzer et al., 2017; Panitz, …, Sperl et al., submitted; Rief et al., 2015; Rief & Glombiewski, 2016; Rief, …, Sperl, & Teige-Mocigemba, in preparation). Thereby, immunization mechanisms can prevent expectation updates. On the one hand, in the context of resilience, immunization can be an adaptive process. In the etiology of anxiety or stressor-related disorders, immunization against dysfunctional changes in expectations (that are induced by aversive and traumatic experiences) could be a promising way to prevent or to reduce psychopathological symptoms. In manuscript 4 of this thesis, we demonstrated that noradrenaline strengthens fear consolidation. During states of noradrenergic hyperarousal (e.g., in the aftermath of a traumatic event), immunization against exaggerated changes in expectations could be a possibility to prevent the pathogenesis of clinical fear. Future research is required to investigate how immunization against noradrenergic hyperarousal can be achieved (e.g., relaxation techniques, noradrenergic antagonists). Furthermore, the issue of which brain processes are associated with cognitive immunization needs to be clarified. On the other hand, immunization during extinction learning may be maladaptive and hinder beneficial effects of exposure therapy. The combination of exposure interventions with rather cognitive strategies (e.g., exposure instructions focusing on expectation violations, see above) could be a promising way to overcome this kind of immunization (Craske et al., 2018; Rief, …, Sperl, & Teige-Mocigemba, in preparation; Sperl, Körfer et al., in preparation).

While accommodation and immunization are cognitive responses that occur after expectation-violating situational outcomes, *assimilation* and *experimentation* are anticipatory behavioral responses, with the goal to actively influence situational outcome probabilities (Gollwitzer et al., 2017; Panitz, …, Sperl et al., submitted; Rief et al., 2015; Rief & Glombiewski, 2016; Rief, …, Sperl, & Teige-Mocigemba, in preparation). The goal of assimilation is to generate expectation-confirming information or to avoid expectation-violating information (Panitz, …, Sperl et al., submitted). In a similar way, experimentation aims to obtain new expectation-relevant information unbiased from preexisting expectations (Panitz, …, Sperl et al., submitted). In typical fear conditioning experiments, participants are relatively passively exposed to situations (e.g., CS and

US presentations) – which is in marked contrast to real-life situations, where individuals (e.g., patients with anxiety disorders) actively select or avoid specific situations. A "lack of experimentation" could be critical for the maintenance of anxiety and fear-related disorders. Novel fear conditioning paradigms should better include active selection components. As an example, participants could be able to actively choose between different CS+/CS- related contexts or to avoid/stop CS and US presentations. Previous studies (including most studies of this thesis) have used neuroscientific methods (e.g., EEG and fMRI) to study neural responses that are mainly related to simple CS *processing*. To further improve the ecological validity of fear conditioning paradigms, future research is required to elucidate brain mechanisms that are relevant for the *selection* of CS+/CS- related situations.

3.5 Conclusion

In summary, the present thesis has addressed a broad range of methodological, catecholaminergic, and translational aspects that are important for neuroscientific (in particular, EEG, but also fMRI) fear conditioning research. The results of this thesis demonstrate that white noise bursts may be particularly well suited for conditioning paradigms that include EEG recordings (**manuscript 1**). For the first time, we have shown that *de novo* fear conditioning can be caused by aversive imagery, in the total absence of any physical or observed aversive stimulation (**manuscript 2**). Furthermore, we have proposed a novel sequential-set fear conditioning paradigm, which allowed us to assess learning dynamics of EEG responses toward threat (**manuscript 3**). A special focus lies on the investigation of associations between catecholaminergic neurotransmitters (noradrenaline, dopamine) and fear/extinction recall (**manuscripts 4 and 5**). Finally, simultaneous EEG-fMRI recordings have been applied to illustrate the interplay between prefrontal theta oscillations and subcortical amygdala activity in humans (**manuscript 6**). As highlighted above, the conceptualization of conditioning from an "expectation" perspective may offer new paths in neuroscientific fear conditioning research.

4 Empirical Studies: Manuscripts 1–6

4.1 Manuscript 1:

A Pragmatic Comparison of Noise Burst and Electric Shock Unconditioned Stimuli for Fear Conditioning Research With Many Trials

Sperl, M. F. J., Panitz, C., Hermann, C., & Mueller, E. M. (2016). A pragmatic comparison of noise burst and electric shock US for fear conditioning research with many trials. *Psychophysiology*, *53*, 1352–1365. https://doi.org/10.1111/psyp.12677

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A pragmatic comparison of noise burst and electric shock unconditioned stimuli for fear conditioning research with many trials

MATTHIAS F. J. SPERL,[a,b] CHRISTIAN PANITZ,[a,b] CHRISTIANE HERMANN,[b] AND ERIK M. MUELLER[a,b]

[a]Faculty of Psychology, Personality Psychology and Assessment, University of Marburg, Marburg, Germany
[b]Faculty of Psychology and Sports Science, Clinical Psychology and Psychotherapy, University of Giessen, Giessen, Germany

Abstract

Several methods that are promising for studying the neurophysiology of fear conditioning (e.g., EEG, MEG) require a high number of trials to achieve an adequate signal-to-noise ratio. While electric shock and white noise burst are among the most commonly used unconditioned stimuli (US) in conventional fear conditioning studies with few trials, it is unknown whether these stimuli are equally well suited for paradigms with many trials. Here, $N = 32$ participants underwent a 260-trial differential fear conditioning and extinction paradigm with a 240-trial recall test 24 h later and neutral faces as conditioned stimuli. In a between-subjects design, either white noise bursts ($n = 16$) or electric shocks ($n = 16$) served as US, and intensities were determined using the most common procedure for each US (i.e., a fixed 95 dB noise burst and a work-up procedure for electric shocks, respectively). In addition to differing US types, groups also differed in closely linked US-associated characteristics (e.g., calibration methods, stimulus intensities, timing). Subjective ratings (arousal/valence), skin conductance, and evoked heart period changes (i.e., fear bradycardia) indicated more reliable, extinction-resistant, and stable conditioning in the white noise burst versus electric shock group. In fear conditioning experiments where many trials are presented, white noise burst should serve as US.

Descriptors: Fear conditioning, Fear extinction, Unconditioned stimulus (US), Electric shock, White noise burst

Experimental studies investigating underlying mechanisms of anxiety disorders and their treatment often use classical fear conditioning and extinction paradigms (Antov, Melicherová, & Stockhorst, 2015; Britton, Evans, & Hernandez, 2014; Duits et al., 2015; Lissek et al., 2008; Wang et al., 2015). After pairing a conditioned stimulus (CS) with an aversive unconditioned stimulus (US) during a conditioning procedure, the CS elicits a conditioned response (CR), which is similar to the spontaneous reaction (unconditioned response, UR) that typically follows the US (LeDoux, 2000). Conversely, extinction learning describes the learning process when the strength of the CR decreases as the CS is presented without the US after conditioning (Furini, Myskiw, & Izquierdo, 2014).

Electro- and magnetophysiological methods that are promising for studying the neurophysiology of fear conditioning and extinction (i.e., ERP or event-related magnetic field [ERMF] components; Moses et al., 2007; Pizzagalli, Greischar, & Davidson, 2003; Stolarova, Keil, & Moratti, 2005), oscillatory neuronal activity (Balderston, Schultz, Baillet, Helmstetter, & Barnes, 2014; Mueller, Panitz, Hermann, & Pizzagalli, 2014), and coupling between electro-/magnetoencephalography (EEG/MEG) and other physiological variables (Moratti & Keil, 2005; Mueller, Stemmler, & Wacker, 2010; Panitz, Hermann, & Mueller, 2015) require a high number of trials to achieve an adequate signal-to-noise ratio (Huffmeijer, Bakermans-Kranenburg, Alink, & van Ijzendoorn, 2014; Miskovic & Keil, 2012; Steinberg, Bröckelmann, Rehbein, Dobel, & Junghöfer, 2013). According to multiple meta-analyses and reviews, electric shocks and white noise bursts are two of the most frequently used USs in human fear conditioning paradigms (Duits et al., 2015; Fullana et al., 2015; Hofmann, de Houwer, Perugini, Baeyens, & Crombez, 2010; Lissek, Powers et al., 2005; Mechias, Etkin, & Kalisch, 2010; Shechner, Hong, Britton, Pine, & Fox, 2014). While there have been various studies comparing different US types in animals and humans using fewer conditioning trials than would be required in typical EEG/MEG studies (Busch & Evans, 1977; Glenn, Lieberman, & Hajcak, 2012; McEchron, McCabe, Green, Llabre, & Schneiderman, 1992; Murray & Carruthers, 1974; Neumann & Waters, 2006), it remains unclear whether electric shocks or loud white noise bursts are more suitable for studying fear conditioning and extinction when the paradigm entails many conditioning trials. Accordingly, EEG and MEG

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Address correspondence to: Erik M. Mueller, Faculty of Psychology, Personality Psychology and Assessment, University of Marburg, Gutenbergstr. 18, 35032 Marburg, Germany. E-mail: erik.mueller@staff.uni-marburg.de

PSYCHOPHYSIOLOGY

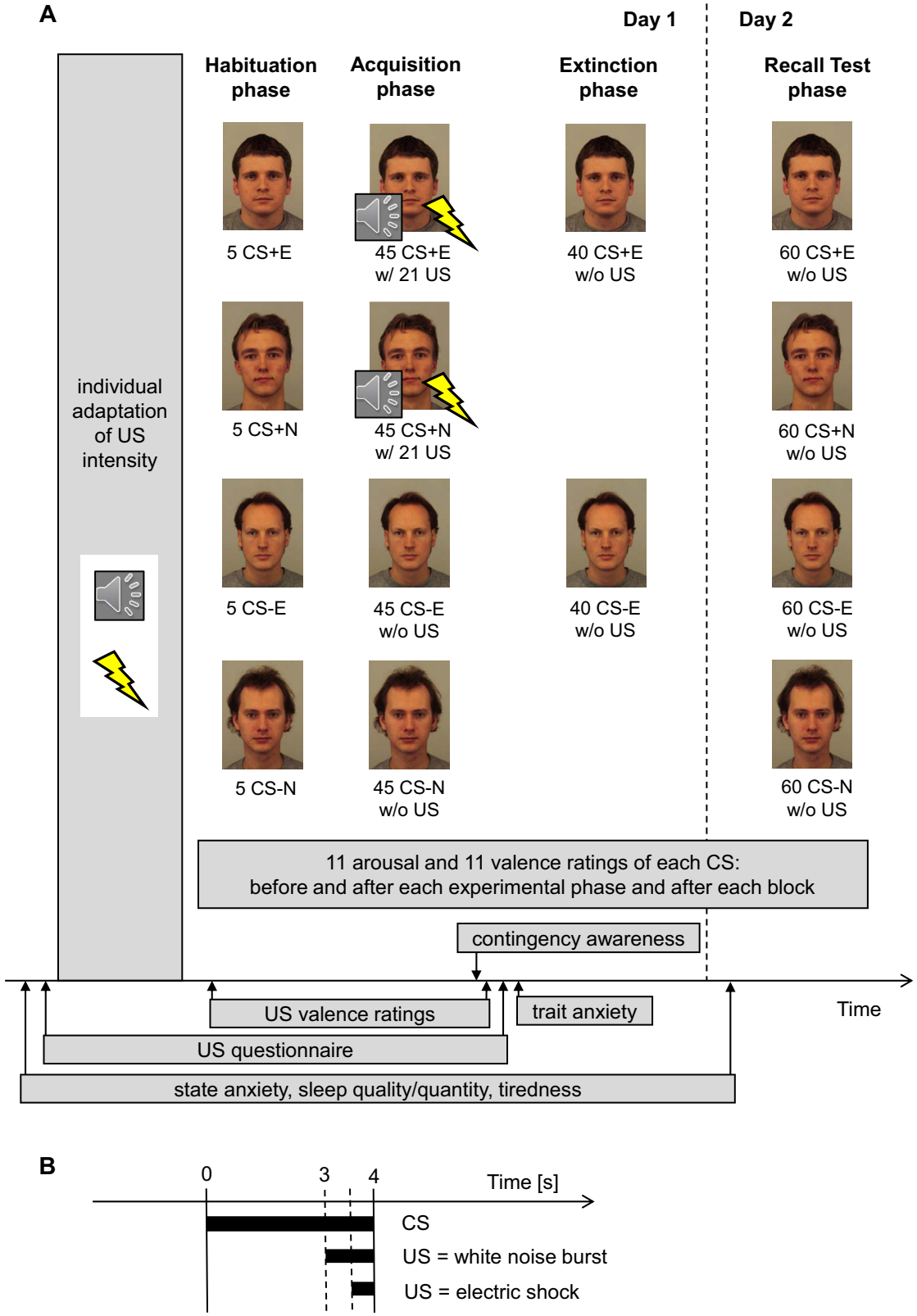

Figure 1. Schematic depiction of the experimental protocol. A: The number and types of stimuli presented during the experimental phases. Face stimuli are taken from Lundqvist et al. (1998), ids: AM10NES, AM13NES, AM31NES, BM08NES. B: Trial structure. CS+ were reinforced with an aversive US (w/) with a contingency of 46.67%. CS− were never paired with a US (w/o). Extinguished stimuli (CS+/−E) were presented during all phases, while nonextinguished stimuli (CS+/−N) were not presented during extinction phase.

researchers are often faced with the rather pragmatic question of which US they should use for their fear conditioning studies. It is a pragmatic question, because it is often not the primary interest to understand the mechanisms that make one US more suitable than the other (e.g., mechanisms such as belongingness, habituation, etc., which is a highly relevant field of research in itself; Öhman & Mineka, 2001). Instead, EEG/MEG research on fear conditioning often focuses on brain mechanisms related to CS processing (Miskovic & Keil, 2012), which makes the selection of an appropriate US crucial, but not central with regard to the study hypotheses.

The appropriateness of a US can be evaluated by considering the strength of the CR. Investigating the physiological basis or moderating mechanisms of fear learning and its association with anxiety disorders requires a successful acquisition of CRs. In theory, an appropriate US should be perceived as unpleasant (Neumann & Waters, 2006; Neumann, Waters, Westbury, & Henry, 2008; Pine et al., 2001), potent (Britton, Lissek, Grillon, Norcross, & Pine, 2011), subjectively meaningful (Kunze, Arntz, & Kindt, 2015), and belonging to the CS (Garcia & Koelling, 1966) in order to obtain successful fear conditioning.

In light of these criteria, it is difficult to decide whether an electric shock or white noise burst as US would result in superior conditioning when many conditioning trials are used. First, the common usage of electric shock to create a threatening context (Nelson, Hajcak, & Shankman, 2015; Schmitz & Grillon, 2012; Weymar, Bradley, Hamm, & Lang, 2013) and of white noise burst to trigger a defensive startle reflex (Blanch, Balada, & Aluja, 2014; Lissek, Baas et al., 2005; Poli & Angrilli, 2015) underlines that both stimuli elicit an aversive response. Second, the intensity of both stimuli is strong enough for fear conditioning with few trials, as both are successfully used in conventional conditioning studies (e.g., Lissek, Powers et al., 2005), but it is unknown whether this also holds for many conditioning trials. Third, for ensuring personal meaningfulness of the US (Kunze et al., 2015), studies on fear conditioning with an electric shock and white noise burst as US usually use different strategies. In studies using an electric shock as US, work-up procedures often are used such that participants select a US intensity that they experience as "unpleasant/annoying but not painful" (Lipp, Kempnich, Jee, Arnold, & Sakakibara, 2014; Merz, Stark, Vaitl, Tabbert, & Wolf, 2013; Orr et al., 2000). In contrast, studies using white noise bursts utilize a single US intensity for all subjects, although the intensity may be reduced for subjects who experience it as too loud (Critchley, Mathias, & Dolan, 2002; Mueller et al., 2014; Peri, Ben-Shakhar, Orr, & Shalev, 2000). Fourth, a comparable degree of survival relevance can be expected for both US types: Hamm, Vaitl, and Lang (1989) showed that a 100 dB tone US and an electric shock US revealed medium to high belongingness ratings with angry faces as CS, being slightly higher for an electric shock US. In sum, theoretical considerations suggest that both US types are potentially suited for fear conditioning with many trials, but they do not provide unequivocal guidance for choosing one US over the other.

The objective of the present study was to examine which US type is better suited for fear conditioning with many trials, under the assumption that standard protocols are used to determine US strength. The fear conditioning study reported here entailed a 2-day fear conditioning and extinction protocol that allowed us to not only study within-session fear conditioning and extinction, but also to study the recall of conditioned and extinguished fear on the second day (Milad et al., 2007; Mueller et al., 2014). Furthermore, the timing and number of trials (Huffmeijer et al., 2014; Miskovic & Keil, 2012; Steinberg et al., 2013) were adapted in order to be sufficient for using electromagnetic recordings. For this paradigm, a "more suitable" US should be characterized by (a) stronger fear conditioning, (b) greater resistance to extinction, and (c) better Day 2 recall of conditioned fear, indexed by physiological responses and subjective ratings. Due to rather similar characteristics of both US types, we did not have an a priori hypothesis regarding which US type would be more suitable.

Method

Participants

In total, 32 students of Justus Liebig University Giessen ($n = 10$ psychology students and $n = 22$ students of sports science; mean age = 22.88 years, $SD = 2.61$ years; 12.5% males) participated in this study for partial fulfillment of course credit. Participants were randomly assigned to two groups of $n = 16$, which differed in the US type that was used for conditioning: electric shock or white noise burst.

Exclusion criteria were use of either prescription drugs or illegal drugs. In addition, none of the subjects reported a history of mental, neurological, or cardiovascular disorders. The study protocol was approved by the ethics committee of the German Psychological Society.

Stimuli and Experimental Paradigm

All subjects took part in a 2-day fear conditioning and extinction paradigm (see Figure 1). On Day 1, the habituation, acquisition, and extinction phases took place. On Day 2 (approximately 24 h after Day 1 testing), recall of conditioned and extinguished fear was tested. The experimental paradigm and stimuli were similar to the one used in a previous study on electrophysiological correlates of conditioned and extinguished fear (Mueller et al., 2014).

Unconditioned stimuli. In the electric shock group, the US was a 500-ms multipulse percutaneous stimulation delivered from a constant current stimulator (DS7A; Digitimer Ltd., UK; 400 V maximal voltage) via two steel disk electrodes of an 8-mm diameter and 23 mm apart. One shock consisted of six single pulses with a duration of 2 ms each and an interval of 100 ms between two pulse onsets. Electrodes were fixed to the inside of the left forearm, about 11 cm from the carpus. During a work-up procedure, electrical stimuli at increasing intensities were presented until they were perceived as "highly annoying but not painful" ($M = 1.69$ mA; $SD = 0.91$ mA). This self-reported level of sensation was used, as it is highly common in fear conditioning studies (Coppens, Spruyt, Vandenbulcke, van Paesschen, & Vansteenwegen, 2009; Martínez, Franco-Chaves, Milad, Quirk, & Felmingham, 2014; Weike, Schupp, & Hamm, 2007). In the white noise burst group, the US consisted of a loud 95 dB white noise burst (duration: 1,000 ms), which was presented over headphones (HD 380 pro; Sennheiser GmbH & Co. KG, Germany). If subjects perceived the 95 dB burst as too loud, sound pressure level was reduced to 92 dB (18.75% of all participants in this group).

Conditioned stimuli. Four male Caucasian faces with a neutral expression (Lundqvist, Flykt, & Öhman, 1998; ids: AM1ONES, AM13NES, AM31NES, BM08NES) served as CS. There were two different CS+ and two different CS−. The assignment of face stimuli to CS types was counterbalanced. All faces were presented

in color on a black background for 4 s with a jittered intertrial interval of 7–9 s (defined as CS offset to CS onset). Presentation of the CSs was preceded by a centered fixation cross (1 s). All faces (size: 11 × 16 cm) were shown on a 17-in. computer monitor placed approximately 50 cm in front of the subject.

Participants were instructed that the US would not be presented during the habituation phase, but that it "may occur" during all other experimental phases. In reality, the US was only presented during acquisition phase. The shock electrodes or headphones were attached during all phases.

The habituation phase consisted of one block of 20 trials, in which each CS was presented five times in random order. The subsequent acquisition phase entailed three blocks with each CS presented 15 times. Both CS+ coterminated with the US at a partial reinforcement rate of 46.67%. US presentation started 3.5 s after CS onset in the electric shock group and 3.0 s after CS onset in the white noise burst group. Both CS− were never paired with the US. There was an approximate 45-min break after the acquisition phase, during which participants completed different questionnaires. The extinction phase consisted of one block during which only one of the CS+ (CS+E; E = extinguished) and one of the CS− (CS−E) were presented 40 times. The other two CSs (CS+N, CS−N; N = nonextinguished) as well as the US did not occur in the extinction phase. On Day 2, all CSs were presented during the recall test phase 20 times in each of three blocks. The US was not presented before or during the recall test phase. By comparing extinguished and nonextinguished CSs (CS+/−E vs. CS+/−N), recall of extinction learning can be distinguished from recall of conditioning learning.

Subjective Ratings

Conditioned stimuli. Before and after each experimental phase and after each block, participants were asked to rate perceived arousal (1 = *not arousing*, 5 = *very arousing*) and valence (−2 = *very pleasant*, 2 = *very unpleasan*t) for each CS on a 5-point Likert scale. Before and after the extinction phase, only CS+E and CS-E were evaluated.

After the acquisition phase, awareness of CS-US contingency was assessed on a 4-point Likert scale from "CS is never followed by US" to "CS is always followed by US."

Unconditioned stimuli. Both directly after adjusting US intensity prior to the habituation phase and after the acquisition phase, the US was presented once, and subjects were asked to rate the valence of the US on an 11-point Likert scale (0 = *not unpleasant at all*, 10 = *extremely unpleasant*). Furthermore, a self-developed questionnaire was used to assess participants' expectations (before work-up procedure and habituation phase) and actual experience (after acquisition phase) concerning the affective quality of the US (see online supporting information for items, item difficulty, and item discrimination coefficients). For each subject, the sum score was calculated by summing up the scores of those items with an item discrimination coefficient of at least .30 both before the habituation phase and after the acquisition phase. Two items were not included in the analyses due to low item discrimination coefficients.

Sleep quality, quantity, and tiredness. In order to quantify the long-term stability of the CR, fear and extinction recall was assessed on Day 2. Since previous studies showed links between fear acquisition, subsequent sleep, and fear/extinction recall (Hellman & Abel, 2007; Marshall, Acheson, Risbrough, Straus, & Drummond, 2014; Pace-Schott, Germain, & Milad, 2015a, 2015b), sleep quality and quantity for the preceding night was evaluated at the beginning of both days. Subjects were asked to indicate subjective sleep quality on a 5-point Likert scale from "very good sleep" to "very bad sleep" and sleep quantity (i.e., the number of hours they had slept in the previous night). Furthermore, current subjective tiredness was registered on a 4-point Likert scale from "not tired at all" to "very tired."

Skin Conductance Responses (SCRs) and Evoked Heart Period (HP)

Physiological data (skin conductance and electrocardiogram, ECG) were recorded at a 1024 Hz sampling rate using the Varioport System (Becker Meditec, Germany) in accordance with publication recommendations (Boucsein et al., 2012; Jennings et al., 1981). For SCRs, exosomatic measurement with 0.5 V direct current was used. Ag/AgCl electrodes of a 10-mm diameter filled with isotonic (0.5% NaCl) electrolyte medium were placed at the thenar/hypothenar sites of the nondominant hand using adhesive tape. ECG was recorded using Ag/AgCl electrodes of a 5.5-cm diameter and filled with liquid gel in Lead II configuration (right arm and left leg, ground electrode on left arm). Physiological data were low-pass filtered (100 Hz) online, and afterward a 4th-order Butterworth offline band-pass filter was applied (SCRs: 0.025–1 Hz; ECG: 1–30 Hz). Offline filters were created with the Signal Processing Toolbox 6.15 and applied using the filter.m function in MATLAB 7.12 (MathWorks, USA). For the ECG, R spikes were automatically detected and manually corrected if necessary. Afterward, continuous HP traces were calculated; that is, each time point represents the distance between the pre- and succeeding R spike (in ms). SCR and HP raw data were manually checked for artifacts. Similar to Milad et al. (2007), for each CS trial an SCR and HP score were calculated. Specifically, the mean skin conductance level of a 1-s pre-CS baseline was subtracted from the peak response within the time window of 6 s after the CS onset. Individual SCR values were normalized by dividing the original SCR value by the individual's maximum SCR within each experimental phase (Lykken & Venables, 1971). Conversely, the HP at stimulus onset was subtracted from the mean HP within the time window from 2 s to 5 s relative to the CS onset (Mueller et al., 2010; Panitz et al., 2015). This time window represents a relatively large deceleration of the HP typically found in the cardiac fear response following CS onset (Lipp, 2006). For the acquisition phase, only unreinforced CS+ trials were included for calculating these mean scores. Outliers of mean SCR and HP scores for each participant (averaged over all trials and conditions and over acquisition, extinction, and recall test phases) were identified using the box plots outlier analysis implemented in SPSS 22 for Windows (IBM, USA). Subjects with an overall mean score more than three times the interquartile range from the upper and lower quartile, respectively, were defined as outliers. With this definition, a total of three subjects were excluded from the SCR analyses, whereas no subject was excluded from the HP analyses.

Measures of Individual Differences in State/Trait Anxiety and Neuroticism

To control for individual differences in anxiety and neuroticism, personality questionnaires were administered. State and trait anxiety was assessed using the German version (Laux, Glanzmann, Schaffner, & Spielberger, 1981) of the State Trait Anxiety

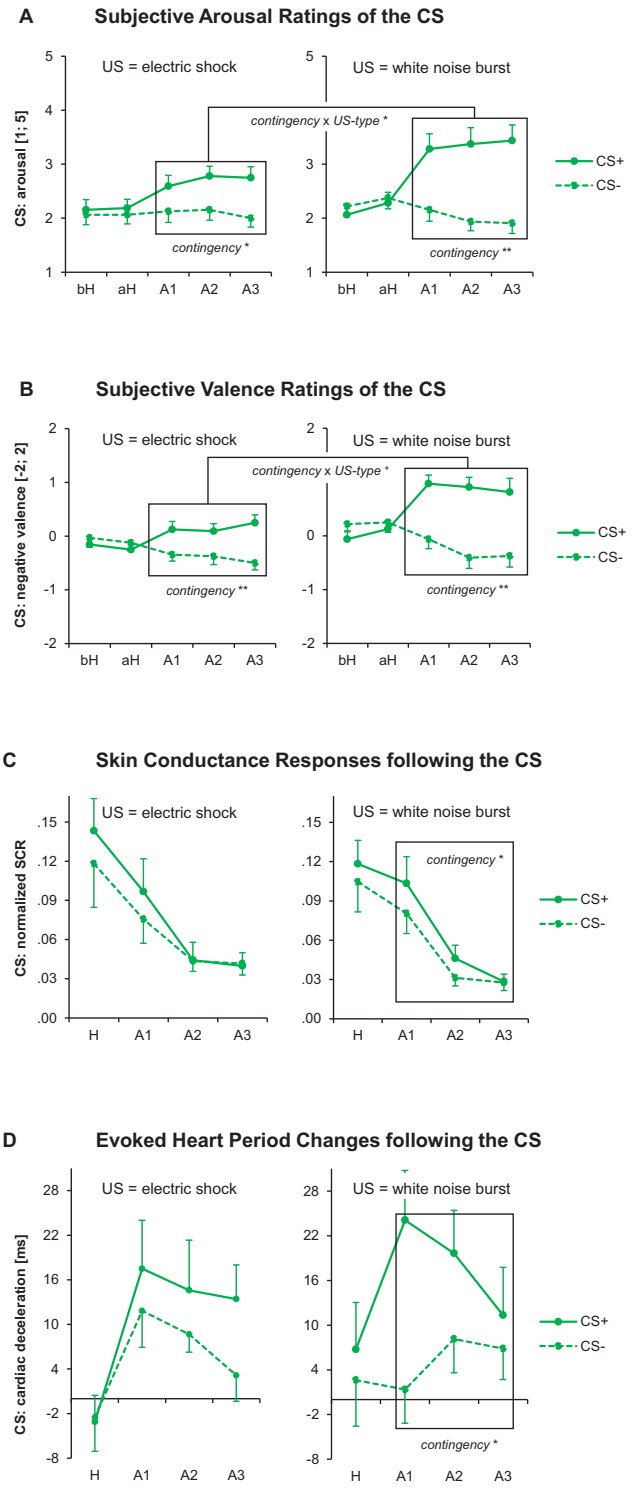

Figure 2. Subjective CS arousal ratings, $M \pm SEM$; 1 = *not arousing*, 5 = *very arousing* (A); subjective CS valence ratings, $M \pm SEM$; −2 = *very pleasant*, 2 = *very unpleasant* (B); normalized CS-evoked SCR amplitudes, $M \pm SEM$ (C); and CS-evoked HP changes (D) during habituation and acquisition phases (Day 1). bH/aH = before/after habituation phase (arousal/valence); H = during habituation phase (SCR, HP); A1, etc. = after (arousal/valence), respectively, during (SCR, HP) acquisition block 1, etc. $^{+}p < .10$; $^{*}p < .05$; $^{**}p < .01$.

Inventory (STAI; Spielberger, Gorsuch, & Lushene, 1970). Additionally, the German version (Ostendorf & Angleitner, 1994) of the neuroticism-anxiety scale of the Zuckerman-Kuhlman Personality Questionnaire (ZKPQ; Zuckerman, Kulhman, Joireman, Teta, & Kraft, 1993) was applied.

Statistical Analysis

In order to examine the impact of US group on differential fear conditioning and extinction, three-way mixed-model analyses of variance (ANOVAs) with contingency (CS+, CS−) and time as repeated measures factors as well as US type (electric shock, white noise burst) as a between-subjects factor were carried out. The factor time referred to each rating of arousal/valence or to the mean SCR/HP score in a block. Each phase of the experiment (habituation, acquisition, extinction, recall test) was analyzed separately for all four dependent variables (arousal and valence ratings, SCR, HP). For acquisition and recall test phases, a SCR/HP mean score was calculated for each CS type and block by averaging across trials of one block. Because there was only one block in the extinction phase, the time factor for SCR and HP analyses in this phase reflected the mean of the first 10 trials and of the last 10 trials. For analyses of all dependent variables in the recall test phase, extinction (CS+/−E, CS+/−N) was included as an additional repeated measures factor. Self-report ratings of the US (US valence, US questionnaire) were analyzed with ANOVAs with time as repeated measures factor and US type as between-subjects factor. In addition, potential influences of state and trait anxiety and neuroticism on fear acquisition were tested. For the state and trait anxiety scales of the STAI and the neuroticism-anxiety scale of the ZKPQ, sum scores were split down the median. For each scale and each dependent variable, the ANOVAs for the acquisition phase were performed again and included the new dichotomized variable for anxiety and neuroticism as additional between-subjects factor. For comparing contingency awareness between the two US groups, an ANOVA with contingency as repeated measures factor and US type as between-subjects factor was performed. Significant interactions and main effects were further analyzed using follow-up ANOVAs and *t* tests when appropriate. When the sphericity assumption was not met, the Greenhouse-Geisser (1959) correction was used. For statistical significance, $p < .05$ was required. All analyses were performed using statistical packages implemented in SPSS 22 for Windows (IBM, USA).

Results

Habituation Phase (Day 1)

Arousal ratings before and after habituation phase displayed no significant main effects or interactions ($ps \geq .175$). Valence ratings revealed that CSs were generally rated as significantly more unpleasant in the white noise burst group compared to the electric shock group even before acquisition, $F(1,30) = 5.84$, $p = .022$, $\eta_p^2 = .163$. SCR amplitudes ($ps \geq .378$) and HP changes ($ps \geq .149$) showed no significant effects (see Figure 2).

Acquisition Phase (Day 1)

Arousal ratings. The Contingency × Time × US Type ANOVA revealed a main effect of contingency, indicating that arousal ratings of the CS+ were significantly greater than those of the CS−, $F(1,30) = 34.12$, $p < .001$, $\eta_p^2 = .532$. A trend for a Contingency × Time interaction showed a stronger CR at the end versus beginning of acquisition phase, $F(2,60) = 3.16$, $p = .050$, $\eta_p^2 = .095$. Importantly, there was also a significant US Type (electric shock,

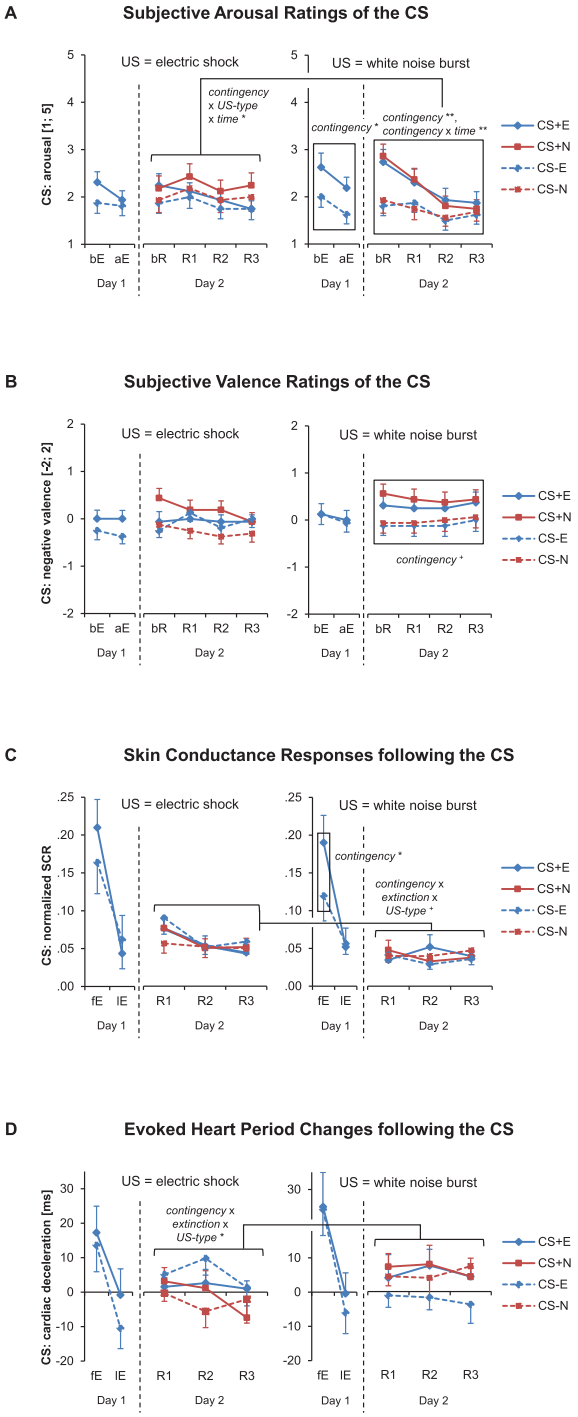

Figure 3. Subjective CS arousal ratings, $M \pm SEM$; 1 = *not arousing*, 5 = *very arousing* (A); subjective CS valence ratings, $M \pm SEM$; −2 = *very pleasant*, 2 = *very unpleasant* (B); normalized CS-evoked SCR amplitudes, $M \pm SEM$ (C); and CS-evoked HP changes (D) during extinction (Day 1) and recall test (Day 2) phases. bE/aE = before/after extinction phase; fE/lE = during the first/last ten extinction trials; R1, etc. = after (arousal/valence), respectively, during (SCR, HP) recall test block 1, etc. $^{+}p < .10$; $*p < .05$; $**p < .01$.

white noise burst) × Contingency (CS+, CS−) interaction, indicating that the differential response to CS+ versus CS− was significantly larger in the white noise burst group (mean difference of arousal ratings on a 5-point Likert scale = 1.36; SD = 0.25) than in the electric shock group (mean difference = 0.61; SD = 0.22), $F(1,30) = 4.90$, $p = .035$, $\eta_p^2 = 140$ (see Figure 2A). No other main effects or interactions were significant ($ps \geq .236$). To additionally probe whether successful fear conditioning could be observed in both US groups individually (electric shock, white noise burst), separate Contingency × Time ANOVAs were performed within each US group. These analyses showed that the CS+ was rated as significantly more arousing than the CS− in both the electric shock, $F(1,15) = 7.53$, $p = .015$, $\eta_p^2 = .334$, and white noise burst, $F(1,15) = 28.81$, $p < .001$, $\eta_p^2 = .658$, group.

Valence ratings. During the acquisition phase, a significant main effect for US type indicated higher ratings in the white noise burst versus shock group, $F(1,30) = 9.59$, $p = .004$, $\eta_p^2 = .242$. Further, the CS+ was rated as significantly more unpleasant than the CS− across groups, $F(1,30) = 25.97$, $p < .001$, $\eta_p^2 = .464$. Moreover, there was a statistically nonsignificant trend (interaction US Type × Contingency, $F(1,30) = 3.24$, $p = .082$, $\eta_p^2 = .098$; Figure 2B) toward a greater CR when using white noise burst as US (mean difference of valence ratings on a 5-point Likert scale = 1.18; SD = 0.29) compared to electric shock (mean difference = 0.56; SD = 0.18). There was also a trend for a main effect of time, $F(2,60) = 2.71$, $p = .075$, $\eta_p^2 = .083$. Exploratory analyses within US groups confirmed that conditioning was successful in both groups (electric shock: $F(1,15) = 9.63$, $p = .007$, $\eta_p^2 = .391$; white noise burst: $F(1,15) = 16.56$, $p = .001$, $\eta_p^2 = .525$).

Skin conductance responses. The three-way ANOVA revealed a significant main effect for the factor time, $F(2,54) = 16.46$, $p < .001$, $\eta_p^2 = .379$. There was no significant main effect or interaction involving contingency or US type, but there was a trend toward a contingency main effect, $F(1,27) = 3.19$, $p = .085$, $\eta_p^2 = .106$. Although the three-way ANOVA showed no significant US Type × Contingency interaction ($p = .573$), we computed separate two-way ANOVAs within the US groups to probe whether there was any evidence for successful conditioning in each condition. These analyses showed a significant main effect of contingency in the white noise burst group, $F(1,13) = 5.86$, $p = .031$, $\eta_p^2 = .311$, but not in the electric shock group, $F(1,14) = 0.508$, $p = .488$, $\eta_p^2 = .035$ (see Figure 2C). Moreover, in both groups, significant main effects of time were observed (electric shock: $F(2,28) = 6.96$, $p = .014$, $\eta_p^2 = .332$; white noise burst: $F(2,26) = 9.40$, $p = .004$, $\eta_p^2 = .420$).

Heart period. A significant main effect for the factor contingency showed a stronger decelerative component following the CS+ compared to the CS−, $F(1,30) = 8.42$, $p = .007$, $\eta_p^2 = .219$. Despite the absence of significant effects concerning the US type factor ($ps \geq .139$), results of separate two-way ANOVAs for both US groups converge with the SCR findings reported above (see Figure 2D): A significant main effect for the contingency factor representing successful fear conditioning was only present in the white noise burst group, $F(1,15) = 5.62$, $p = .032$, $\eta_p^2 = .273$, but absent in the electric shock group, $F(1,15) = 2.82$, $p = .114$, $\eta_p^2 = .158$.

Extinction Phase (Day 1)

Mean subjective arousal and valence ratings, normalized SCR amplitudes following the CS, as well as CS-evoked HP changes during extinction and recall test phases are shown in Figure 3.

Arousal ratings. The Contingency × Time (before vs. after extinction) × US Type ANOVA on arousal ratings indicated that

the CS+ was evaluated as significantly more arousing than the CS− (main effect contingency: $F(1,30) = 7.79$, $p = .009$, $\eta_p^2 = .206$). Moreover, arousal ratings were significantly lower after than before extinction (main effect time, $F(1,30) = 5.18$, $p = .030$, $\eta_p^2 = .147$). There were no other significant interactions or main effects ($ps \geq .161$). Separate two-way ANOVAs for both US groups revealed that the CS+ was rated as significantly more arousing than the CS− only in the white noise burst group, $F(1,15) = 6.99$, $p = .018$, $\eta_p^2 = .318$ (see Figure 3A). Conversely, in the electric shock group, no significant main effect of contingency was found, $F(1,15) = 1.65$, $p = .218$, $\eta_p^2 = .099$. These results show that a CR was successfully recalled with regard to arousal ratings in the noise burst but not in the shock group.

Valence ratings. The Contingency × Time × US Type ANOVA on valence ratings showed a trend toward more negative ratings in the white noise burst group (main effect US type, $F(1,30) = 3.25$, $p = .081$, $\eta_p^2 = .098$). Moreover, there was also a trend toward a main effect of time, $F(1,30) = 3.82$, $p = .060$, $\eta_p^2 = .113$. There were no more significant effects ($ps \geq .520$). Separate two-way ANOVAs for both US groups revealed no main effects or interactions involving contingency ($ps > .333$). In the electric shock group, CS-associated unpleasantness decreased from before to after extinction (main effect time: $F(1,15) = 4.62$, $p = .048$, $\eta_p^2 = .0236$).

Skin conductance responses. The Contingency × Time × US Type ANOVA on SCRs revealed a significant main effect of time, $F(1,27) = 17.45$, $p < .001$, $\eta_p^2 = .393$, and a Time × Contingency interaction, $F(1,27) = 4.59$, $p = .041$, $\eta_p^2 = .145$, indicating a significant reduction of differential SCRs from the beginning (first 10 trials, CS+ vs. CS−: $t(28) = 2.48$, $p = .019$, $d = 0.461$) to the end of extinction (last 10 trials, CS+ vs. CS−: $t(28) = 0.49$, $p = .625$, $d = 0.092$). There were no other significant main effects or interactions ($ps > .183$). As there were no differential fear reactions at the end of extinction, we performed separate t tests for both US groups only for the beginning of extinction to probe whether a successful short-term fear conditioning recall could be observed within groups. Within the electric shock group, SCRs did not significantly differ between CS+ and CS−, $t(14) = 1.32$, $p = .208$, $d = 0.341$. However, within the noise burst group, SCRs were significantly increased for CS+ vs. CS−, $t(13) = 2.23$, $p = .044$, $d = 0.597$ (Figure 3C).

Heart period. Analyses of HP changes in response to the CS during extinction phase revealed a significant main effect of time, indicating a weaker deceleration component to all CS types at the end versus beginning of extinction phase, $F(1,30) = 16.89$, $p < .001$, $\eta_p^2 = .360$.

Recall Test Phase (Day 2)

Arousal ratings. The Contingency × Extinction × Time × US Type ANOVA revealed a significant three-way interaction of contingency, time, and US type, $F(3,90) = 2.95$, $p = .047$, $\eta_p^2 = .089$. Follow-up ANOVAs for each time of measurement showed a significant US Type × Contingency interaction only before the first block, $F(1,30) = 4.72$, $p = .038$, $\eta_p^2 = .136$, but not later ($ps \geq .202$). This interaction indicated that the recalled CR was significantly larger in the white noise burst group than in the electric shock group before the beginning of the recall test phase (Figure 3A). Further, the four-way ANOVA also showed significant main effects of time, $F(3,90) = 11.44$, $p < .001$, $\eta_p^2 = .276$, contingency, $F(1,30) = 12.00$, $p = .002$, $\eta_p^2 = .286$, as well as significant interactions of contingency and time, $F(3,90) = 7.47$, $p < .001$, $\eta_p^2 = .199$, and of US type and time, $F(3,90) = 4.11$, $p = .017$, $\eta_p^2 = .120$. There was also a trend toward an Extinction × Time × US Type interaction, $F(3,90) = 2.53$, $p = .062$, $\eta_p^2 = .078$. Separate Time × Contingency × Extinction ANOVAs for each US group demonstrated that Day 1 conditioning was only successfully recalled in the white noise burst group (see Figure 3A): Ratings of the CS+ were significantly higher than those of the CS− across all recall test trials when white noise burst served as US, $F(1,15) = 9.82$, $p = .007$, $\eta_p^2 = .396$. The main effect of time (Time 1, 2, 3, and 4) was also significant, $F(3,45) = 14.22$, $p < .001$, $\eta_p^2 = .487$, and a significant interaction between the factors time and contingency indicated an additional extinction effect during the recall test phase, $F(3,45) = 9.23$, $p < .001$, $\eta_p^2 = .381$, in the white noise burst group. Subsequent pairwise comparisons showed that CS+ and CS− ratings only differed significantly before, $t(15) = 4.29$, $p = .001$, $d = 1.074$, and after, $t(15) = 2.40$, $p = .030$, $d = 0.601$, the first block of the recall test phase. Ratings of CS+ and CS− differed by trend after the second block, $t(15) = 2.11$, $p = .052$, $d = 0.528$, but were comparable after the third block ($p = .173$). In contrast, the three-way ANOVA for the electric shock group only showed trends for an extinction main effect, $F(1,15) = 3.78$, $p = .071$, $\eta_p^2 = .201$, and an Extinction × Time interaction, $F(3,45) = 2.44$, $p = .077$, $\eta_p^2 = .140$.

Valence ratings. The four-way ANOVA indicated successful recall of Day 1 conditioning across groups (main effect contingency: $F(1,30) = 5.30$, $p = .028$, $\eta_p^2 = .150$) and a significant interaction of extinction and time, $F(3,90) = 2.95$, $p = .037$, $\eta_p^2 = .090$, but no significant interaction involving US type ($ps \geq .199$). In order to test whether recall of conditioned fear was successful in both groups, two separate ANOVAs were computed: As displayed in Figure 3B, there was a trend for a successful recall of Day 1 conditioning expressed by valence ratings only in the white noise burst group (main effect contingency: $F(1,15) = 4.13$, $p = .060$, $\eta_p^2 = .216$), but not in the electric shock group, $F(1,15) = 1.45$, $p = .248$, $\eta_p^2 = .088$. When the electric shock was used as US, only the Extinction × Time interaction was significant, $F(3,45) = 3.90$, $p = .015$, $\eta_p^2 = .206$.

Skin conductance responses. Analyses of the SCR amplitudes in response to the CS on Day 2 revealed a significant Contingency × Extinction × Time interaction, $F(2,54) = 3.39$, $p = .041$, $\eta_p^2 = .112$. Follow-up Contingency × Extinction ANOVAs showed a significant interaction between these two factors only during the first block, $F(1,28) = 5.57$, $p = .025$, $\eta_p^2 = .166$, but not during the second and third block of the recall test ($ps \geq .226$). These results indicate a successful recall of conditioned fear during the first block for nonextinguished stimuli by trend (CS+N vs. CS−N: $t(28) = 1.87$, $p = .072$, $d = 0.347$) as well as successful recall of extinguished fear (CS+E vs. CS−E: $t(28) = 1.43$, $p = .165$, $d = 0.265$). Moreover, there was a trend for a Contingency × Extinction × US Type interaction, $F(1,27) = 3.92$, $p = .058$, $d = 0.127$.

Heart period. The Contingency × Extinction × Time × US Type ANOVA on evoked HP changes revealed a significant interaction between the factors contingency, extinction and US type, $F(1, 30) = 5.19$, $p = .030$, $\eta_p^2 = .147$, as well as an Extinction × US Type interaction, $F(1,30) = 7.39$, $p = .011$, $\eta_p^2 = .198$. To further investigate the interactions of the four-way ANOVA, differential scores (CS+N vs. CS−N, CS+E vs. CS−E) were computed

to test the recall of the conditioned fear response on Day 2. The recall of the CR represented by those differential scores was significantly stronger in the white noise burst versus electric shock group only for extinguished stimuli, $t(30) = 2.27$, $p = .031$, $d = 0.801$, but not for nonextinguished stimuli, $t(30) = 0.08$, $p = .936$, $d = 0.029$ (see Figure 3D). These results suggest that white noise burst leads to a more extinction-resistant conditioned fear response.

US-Ratings, US Questionnaire and Contingency Awareness

The Time × US Type ANOVA on the US valence ratings revealed significant main effects of time, $F(1,30) = 27.90$, $p < .001$, $\eta_p^2 = .482$, and of US type, $F(1,30) = 14.63$, $p = .001$, $\eta_p^2 = .328$, that were further qualified by a significant US Type × Time interaction, $F(1,30) = 23.53$, $p < .001$, $\eta_p^2 = .440$. This interaction indicated a significant decline of US negative valence ratings in the electric shock group, $t(15) = 5.93$, $p < .001$, $d = 1.482$, but not in the white noise burst group, $t(15) = 0.42$, $p = .684$, $d = 0.104$. From another perspective, US negative valence ratings were comparable for the two groups prior to the habituation phase, $t(30) = 1.01$, $p = .324$, $d = 0.357$, but were significantly lower for the electric shock after the acquisition phase, $t(30) = 5.11$, $p < .001$, $d = 1.808$. Similarly, the Time × US Type ANOVA on the US questionnaire revealed higher mean scores in the white noise burst as compared to the electric shock group, $F(1,30) = 6.10$, $p = .019$, $\eta_p^2 = .169$.[1] Finally, contingency awareness of the CS−US relationship after the acquisition phase did not significantly depend on type of US (interaction Contingency × US Type: $F(1,30) = 0.48$, $p = .493$, $\eta_p^2 = .016$; main effect contingency: $F(1,30) = 204.28$, $p < .001$, $\eta_p^2 = .872$) and was given in both groups (both $ps < .001$).

Analysis on Individual Differences in State/Trait Anxiety

In order to probe whether the influence of US type on fear acquisition was modulated by individual differences in state/trait anxiety and neuroticism, secondary ANOVAs for the dependent variables arousal/valence ratings, SCRs, and HP changes were conducted for Day 1 acquisition. Dichotomized trait/state anxiety and neuroticism (STAI, ZKPQ) scores were included as additional between-subjects factor.

Arousal ratings. For arousal ratings of the CS, ANOVAs with dichotomized STAI-trait and ZKPQ-neuroticism-anxiety scores as between-subjects factors did not show threefold interactions between theses sum scores, contingency and US type ($ps \geq .282$). Importantly, Contingency × US Type interactions remained significant ($ps \leq .033$), indicating stronger conditioning with white noise burst, independently of differences in trait anxiety and neuroticism. State anxiety was associated with differences in differential arousal ratings between both US types (interaction Contingency × Block × US Type × STAI-State Anxiety, $F(2,56) = 3.46$, $p = .038$, $\eta_p^2 = .110$). Separate follow-up ANOVAs for both US groups revealed a significant Contingency × Block × STAI-State Anxiety interaction only for electric shocks, $F(2,28) = 4.72$, $p = .017$, $\eta_p^2 = .252$, indicating slower conditioning for subjects with high state anxiety. Conversely, in the white noise burst group, there was no Contingency (× Block) × STAI-State Anxiety interaction ($ps \geq .510$), but a significant contingency main effect, $F(1,14) = 26.89$, $p < .001$, $\eta_p^2 = .658$. Pearson's chi-square test did not show any association between US group and dichotomized STAI-state score, $\chi^2(1, N = 32) = 0.13$, $p > .999$. Thus, individuals in the white noise group were not characterized by low STAI-state scores. Since subjects with high versus low state anxiety showed slow conditioning with electric shocks but not with noise burst, these findings are consistent with better conditioning with white noise US.

Valence ratings. Differential valence ratings of CS revealed stronger (interaction Contingency × US Type) fear conditioning for white noise burst, also when including STAI-State ($p = .076$), STAI-Trait ($p = .086$) and ZKPQ-neuroticism-anxiety ($p = .068$) as additional factors. Of relevance, there was no significant effect involving anxiety/neuroticism ($ps \geq .184$).

Skin conductance responses. For SCRs, there was no significant interaction between contingency, US type and anxiety/neuroticism ($ps \geq .206$). Supporting our previous finding, higher SCRs for CS+ versus CS− (main effect contingency) could only be found for white noise bursts ($ps \leq .037$), but not electric shocks ($ps \geq .488$). Within the white noise burst group, fear acquisition was stronger for subjects with low trait anxiety and neuroticism scores (interaction Contingency × STAI-Trait: $p = .006$; interaction Contingency × ZKPQ-Neuroticism-Anxiety: $p = .015$). Subjects with lower trait anxiety also showed a stronger decline of SCR amplitudes during acquisition phase, independently of US and CS type (interaction Time × STAI-Trait Anxiety, $F(2,56) = 5.63$, $p = .014$, $\eta_p^2 = .167$).

Heart period. There was no effect involving anxiety/neuroticism for HP changes ($ps \geq .173$). Converging with previously reported findings, a stronger heart rate deceleration for CS+ versus CS− was only found for white noise bursts ($ps \leq .038$), but not for electric shocks ($ps \geq .116$).

In sum, subjects showed stronger conditioning with white noise burst also when measures of state and trait anxiety or neuroticism were included into the analyses. In fact, with regard to arousal ratings, only white noise burst produced a robust conditioned response for subjects with low and high state anxiety. None of the other previously in the Results section on Day 1 acquisition reported effects involving US type were qualified by state/trait anxiety.

1. In addition to subjective ratings of the US, SCR amplitudes to the US were analyzed to probe changes in US-evoked SCRs during acquisition phase. A statistically nonsignificant trend for the Time × US Type interaction indicated a stronger reduction of SCRs for the white noise burst compared to the electric shock. Based on previous literature on US responses during fear acquisition, this reveals further evidence for enhanced fear conditioning with white noise burst. Paradoxically, it has been shown that successful conditioning leads to a reduction of the unconditioned electrodermal response (Dunsmoor, Bandettini, & Knight, 2008; Knight, Waters, King, & Bandettini, 2010; Wood, Kuykendall, Ver Hoef, & Knight, 2013), which is referred to as "conditioned diminution of the UR" (Kimble & Ost, 1961; Marcos & Redondo, 1999). As the diminution of the UR was stronger for white noise bursts versus electric shocks, this finding also suggests that white noise burst was associated with stronger fear acquisition. Rust (1976) argues that the electrodermal UR mainly reflects an orienting response that declines as the US is getting less novel and more predictable. In sum, the stronger reduction of US negative valence ratings in the course of acquisition for the electric shock group mainly reflects a reduction of US aversiveness (potentially due to stronger habituation). Conversely, the stronger SCR diminution for the white noise burst can mainly be interpreted in terms of US expectancy, as the US is getting more predictable by the CS. Both less reduction of negative valence ratings as well as SCR diminution indicate better conditioning with white noise burst versus electric shock. However, it should be noted that comparisons of the URs of both US types are limited as white noise bursts and electric shocks are qualitatively different stimuli.

Subjective Sleep Quality, Quantity, and Tiredness

Before habituation phase on Day 1, subjectively reported sleep quality ($p = .668$), quantity ($p = .184$), and tiredness ($p = .540$) did not differ significantly between both US groups. However, before the Day 2 recall test, subjects in the white noise burst group reported significantly worse sleep quality, $t(30) = 2.47$, $p = .019$, $d = 0.874$, and significantly enhanced tiredness, $t(30) = 2.42$, $p = .023$, $d = 0.857$, than subjects in the electric shock group. Moreover, there was a trend for subjects in the white noise burst group to report fewer sleeping hours for the previous night, $t(30) = 1.80$, $p = .082$, $d = 0.636$.

Discussion

The objective of the present study was to identify a suitable US for fear conditioning and extinction protocols with a high number of trials, as these protocols are required for neurophysiological methods (e.g., EEG, MEG) in order to assure an adequate signal-to-noise ratio. Two US types that are commonly used in conventional fear learning studies, electric shock and white noise burst, were directly compared in a 2-day between-groups conditioning and extinction paradigm. Several measures of conditioned fear indicated that subjects exhibited a stronger (SCRs, HP changes, arousal, and valence ratings) and more extinction-resistant (arousal ratings, HP) CR when a white noise burst was used as US as compared to an electric shock US. Moreover, a white noise burst as US resulted in better recall of conditioned fear approximately 24 h later (arousal and valence ratings, HP). Finally, US ratings and a newly developed US questionnaire indicated that the valence of the white noise burst versus electric shock remained more unpleasant throughout the course of conditioning.

Arousal and valence ratings revealed superior fear acquisition for white noise burst versus electric shock as US. Complementing these behavioral findings, SCRs as well as HP changes in the acquisition phase only discriminated CS+ and CS− when the white noise burst served as US. Of relevance, subjects in the white noise burst group showed better fear acquisition even when individual anxiety measures were taken into account. These results converge with previous studies on fear conditioning with many trials that reported successful fear conditioning when using white noise as US (Dolan, Heinze, Hurlemann, & Hinrichs, 2006; Moses et al., 2007; Mueller et al., 2014). Building on early studies using intracranial (Lesse, 1957) and scalp (Flor et al., 1996) recordings to explore electromagnetic brain activity of associative learning processes, EEG and MEG on human fear conditioning have increasingly been used in the last 15 years (Dolan et al., 2006; Pizzagalli et al, 2003; Steinberg et al., 2012). Miskovic and Keil (2012) reviewed many of these studies, which consisted of more acquisition trials than conventional conditioning studies (Büchel & Dolan, 2000). In most of these studies, electric shocks or white noise bursts were used as US. As our findings suggest inferior conditioning effects for electric shocks versus white noise bursts, the usage of electric shocks might have resulted in a reduced power for detecting brain activity that discriminates CS+ and CS−.

Before (arousal ratings, SCRs) and after (arousal ratings) extinction phase, only participants in the white noise burst group, but not in the electric shock group, showed a differential fear response. This observation is not only consistent with the previously described enhanced fear acquisition in the noise burst versus electric shock group, but further converges with previous studies on fear extinction that used many trials and auditory US and observed extinction-resistant differences between CS+ and CS− as assessed with self-reported fear (Ugland, Dyson, & Field, 2013) as well as heart rate responses and electromagnetic correlates (Moratti & Keil, 2005; Regan & Howard, 1995). In contrast, there was no evidence for conditioned fear during or after extinction in the electric shock group. Similarly, other studies on fear extinction, which used many trials and an electric shock as US (Flor et al., 1996; Wik, Elbert, Fredrikson, Hoke, & Ross, 1997) reported that the previously acquired CR was extinguished at the end of the extinction phase. This suggests that, with standard protocols for intensity calibration, conditioned fear may be more labile for electric shock versus noise burst US.

Even approximately 24 h after the acquisition phase, subjective ratings indicated a larger recall of conditioned fear when white noise burst (as opposed to electric shock) served as US. Complementary to this finding, evoked HP changes showed a significantly stronger fear recall in the white noise burst versus electric shock group for previously extinguished stimuli, supporting the hypothesis that fear acquired by white noise burst (vs. electric shock) is more extinction resistant. Unlike HP, differential SCRs were modulated by Day 1 extinction, but not by US type.

Supporting results from analyses of subjective CS ratings and physiological responses, only on Day 2, but not on Day 1, participants in the white noise burst group reported impaired sleep quality and quantity for the previous night compared to participants in the electric shock group. This finding indicates that the white noise US may have impaired the sleep of the subjects during the night after fear acquisition and extinction (Sanford, Suchecki, & Meerlo, 2015). This impact of fear learning on the following sleep period is in line with previous research, which showed a connection between US intensity and subsequent sleep quality (Sturm, Czisch, & Spoormaker, 2013). Further, supporting the habituation hypothesis mentioned above (i.e., worse fear learning may be mediated by stronger habituation for the shock), it has been shown that impaired sleep quality and quantity after fear conditioning was associated with reduced physiological habituation to an electric shock US during acquisition (Spoormaker et al., 2010). Moreover, animal studies reported reduced rapid eye movement (REM) sleep after fear acquisition (Kumar, Jha, & Kline, 2012), possibly mediated by enhanced locus coeruleus activity (Germain, Buysse, & Nofzinger, 2008). Subjective ratings of sleep quality and quantity are therefore supporting our findings that a white noise US led to the formation of a stronger fear memory.

The present study compared noise burst and shock US in the way they are commonly used in fear conditioning studies, and found that white noise bursts produced more reliable CRs than electric shocks when a paradigm with many trials was used. The goal of this study was to provide guidance for researchers when deciding which US to use in EEG or MEG fear conditioning studies rather than uncovering potential underlying causes for the advantage of one US over the other. Nevertheless, one can speculate about potential mechanisms that facilitated fear conditioning with the noise burst. Mechanisms such as a stronger habituation to the electric shock (Çevik, 2014; Dycus & Powers, 2000; Jordan, Todd, Bucci, & Leaton, 2015), the use of different US-calibration procedures for shock versus noise burst (Baker, Mercier, Gabel, & Baker, 1981; Lipp, 2006; Randich & LoLordo, 1979), different US durations (Gallistel & Gibbon, 2000; Lipp, 2006; Marter et al., 2014), different levels of belongingness (Hamm et al., 1989), and the involvement of different neural pathways (LeDoux, 2014) may have contributed to better learning in the noise burst versus shock group.

Given that, initially, the aversiveness of both US types was similar, and the observation that the aversiveness and negative affective quality of the electric shock versus white noise burst declined throughout acquisition, it appears that participants habituated more strongly to the shock than to the noise burst. A stronger habituation would be associated with a lower difference between actual and predicted US intensity and consequently with weaker learning (Rescorla & Wagner, 1972).

Moreover, both US types require different calibration procedures. Whereas institutional review boards usually demand work-up procedures implying gradually increasing shock intensities, acoustic stimuli are typically used with a preset intensity in research. Crucially, the direct role in calibrating and choosing the shock intensity provides participants with a sense of control over the electric shock intensity and delivery, which is absent for the white noise burst.[2] Surprisingly, CSs were generally rated as more unpleasant in the white noise burst group already during habituation phase (i.e., even before the onset of CS−US pairings). This negative priming effect may partially be explained by the different US intensity calibration methods. The bigger lack of control concerning US intensity for white noise burst may have enhanced state anxiety, which may have produced more negative baseline ratings for all CS types (Baas, Milstein, Donlevy, & Grillon, 2006; Grillon & Charney, 2011; Rosen & Schulkin, 1998).

Various animal and human studies have examined the influence of US duration on fear acquisition. Some studies found better conditioning for longer US durations (Ashton, Bitgood, & Moore, 1969; Bitterman, Reed, & Krauskopf, 1952; Burkhardt & Ayres, 1978; Overmier, 1966), but there are also contradictory results (Frey & Butler, 1973; Kawai & Imada, 1996; Marter et al., 2014; Meiselman & Moore, 1965; Tait, Kehoe, & Gormezano, 1983; Wegner & Zeaman, 1958). Consequently, better conditioning with white noise burst could partially be related to a longer US duration and a longer CS−US overlap. However, the aim of the present study was to compare US types with those characteristics as they are typically used in research. The duration of electric shock US is usually varying between a few ms and 500 ms (Lipp, 2006). Given that we used 500 ms (i.e., compared to other studies a relatively long shock in the present study), our measures of the CR with electric shock are rather over- than underestimated with regard to commonly used paradigms. Moreover, 1,000-ms white noise bursts are often used in fear conditioning studies and demonstrate ecological validity for many fear conditioning experiments (Khodam Hazrati, Miskovic, Príncipe, & Keil, 2015; LaBar, LeDoux, Spencer, & Phelps, 1995; Leer & Engelhard, 2015).

It cannot be ruled out that superior fear conditioning with white noise burst may also be due to higher belongingness with neutral faces (Öhman & Mineka, 2001). Nevertheless, based upon the results of Hamm et al. (1989) showing slightly higher belongingness ratings for angry faces CSs with electric shock USs compared to loud tone USs, it is less likely that stronger fear acquisition for white noise was related to different CS−US belongingness.

As the current study did not include any direct measures of brain activity, it remains unclear whether enhanced conditioning with white noise burst was due to stronger neuroanatomical connectivity between visual (faces CSs) and auditory (noise US) cortices. However, a review of Sehlmeyer et al. (2009) gives some descriptive support that the neuronal fear network might be more reliable detected by auditory USs. While 78% of all studies using acoustic USs showed amygdala activity during conditioning, this effect was only present in 45% of studies with tactile USs. Also for anterior cingulate and insula, activity during conditioning could be shown in more studies when acoustic USs (56%) were applied compared to tactile USs (30%).

Despite potential influences of the previously mentioned possible mechanisms for enhanced conditioning with white noise burst, it should be pointed out that it was not the primary goal of the present study to clarify the relative role of these processes. In fact, our aim was to give practical advice for designing fear conditioning paradigms, independently of potential underlying mechanisms. Revealing the influence of those mechanisms would require experimental designs differing from the present study.

It is important to emphasize that the present observations are only valid for paradigms in which many trials are used for conditioning such as EEG/MEG experiments. It cannot be ruled out that an electric shock is more suitable in experiments using fewer trials than typical EEG/MEG studies. In fact, Cook, Hodes, and Lang (1986) described an electric shock as a more powerful reinforcer than noise, when phobia-relevant stimuli served as CS. Additionally, partially supported by our additional analysis on anxiety and neuroticism, Glenn et al. (2012) reported that an electric shock US is potentially more sensitive for detecting individual differences in trait anxiety compared to an acoustic US, which consisted of a fearful face paired with a scream. In addition, it should be mentioned that subjective arousal and valence ratings as well as SCRs and HP changes indicate a stronger acquisition of the CR for white noise burst, although contingency awareness did not differ between the two US groups. Consequently, white noise burst is particularly superior for detecting affective and physiological measures of the CR, in spite of a comparable cognitive awareness of CS−US contingencies.

Some limitations of the present study should be acknowledged. First, it remains unclear if a white noise burst is also superior as a US when physiological methods are used that include unusual experimental contexts, like fMRI or positron emission tomography (PET). For instance, high levels of background noise of these techniques might possibly reduce the aversiveness of the white noise burst. Future studies using methods like fMRI or PET may ultimately answer the question about the utility of both US types during recording of these physiological parameters. Second, for both female and male participants, pictures of male neutral faces served as CS, which reduces the generalizability of the present findings with regard to CS type. Lissek, Powers et al. (2005) compared different CS types in a meta-analysis and found the most substantial effect sizes for fear acquisition when faces served as CS. Notably, many studies on fear conditioning use male faces as CS for both male and female participants (Dunsmoor & LaBar, 2012; Guhn et al., 2012; Pischek-Simpson, Boschen, Neumann, & Waters, 2009; Wieser, Miskovic, Rausch, & Keil, 2014). Moreover, it has been argued that adult male faces are particularly prepared for fear conditioning (Mazurski, Bond, Siddle, & Lovibond, 1996; Öhman & Dimberg, 1978). Nevertheless, future studies should clarify

2. This interpretation is supported by an explorative analysis of the US questionnaire, which included the item "The electric shock/white noise burst is uncontrollable." A Time × US Type ANOVA for this item revealed a main effect for the factor US type, $F(1,30) = 4.98$, $p = .033$, $\eta_p^2 = .142$. White noise burst was perceived as significantly more uncontrollable than electric shocks across both times of measurement. However, it should be considered that subjects completed the questionnaire for the first time even before US calibration. The lack of an US Type × Time interaction ($p = .107$) reflects that enhanced uncontrollability for white noise burst is not only related to different intensity calibration methods, but also to different a priori expectations.

whether the present findings generalize to similar paradigms with other CSs. Third, in light of the dual-process theory of fear learning (den Dulk, Heerebout, & Phaf, 2003; LeDoux, 1995, 2014), explicit declarative fear knowledge should be differentiated from implicit nonconscious fear memory processes. There is evidence that SCRs reflect mainly associative-explicit facets of fear learning (Hamm & Weike, 2005; Sevenster, Beckers, & Kindt, 2014), whereas conditioned startle potentiation rather reflects emotional-implicit aspects (Sevenster et al., 2014; Weike et al., 2007; but see Schultz & Helmstetter, 2010). Because fear-potentiated startle responses were not recorded in our study, it is difficult to state whether our findings generalize to implicit fear memory. By showing fear conditioning effects in contingency-unaware subjects, we previously demonstrated implicit fear conditioning with the noise burst US with the current design (Mueller & Pizzagalli, 2016). Nevertheless, future comparisons of white noise burst and electric shock should also include (nonauditory) startle probes to test whether the white noise burst is also preferable for implicit fear memory studies. Fourth, it should be kept in mind that time windows and peak detection algorithms vary across fear conditioning studies using SCRs (Becker et al., 2013; Büchel, Morris, Dolan, & Friston, 1998; Eckstein et al., 2016; Hermann et al., 2012; Kalisch et al., 2009; Lonsdorf, Haaker, Fadai, & Kalisch, 2014; Milad, Orr, Pitman, & Rauch, 2005; Mueller & Pizzagalli, 2016; Phelps, Delgado, Nearing, & LeDoux, 2004; Vervliet, Vansteenwegen, & Eelen, 2004; Winkelmann et al., 2016). There are slight deviations between the current approach and the recommendations of Boucsein et al. (2012), which may limit the generalizability of our SCR findings. However, in line with arousal/valence ratings and HP analyses, the current measurement approach of SCR was sensitive to discriminate between CS+ and CS− in the white noise, but not the shock group, which supports the validity and sensitivity of the current measurement and analysis pipeline. Finally, the present study compared two USs using experimental protocols that are commonly used in fear conditioning research. It should be noted that any modifications of these protocols (e.g., change in noise burst intensity, shock quality, shock location, or work-up procedure) may affect the observed pattern, and more work is needed to further identify the optimal conditions for human fear conditioning research with many trials. It is important to point out that stronger conditioning in the white noise group cannot be attributed only to the white noise burst itself, but also to other US-associated procedure characteristics (e.g., somatosensory vs. auditory stimulation, work-up procedure for the electric shock, US intensity, duration of the US, onset of the US after CS onset). Nevertheless, we believe that the present comparison of two very commonly used protocols provides important information that may help researchers when planning future fear conditioning studies.

In conclusion, the overall findings of the present study showed that white noise bursts yield more robust conditioning effects as compared to an electric shock as US when many trials and standard protocols to determine US intensity are used. Specifically, white noise burst resulted in a stronger, more extinction-resistant, and more stable acquisition of fear. Regardless of the exact underlying mechanisms, the current findings suggest that future studies applying psychophysiological methods that require a high number of trials (e.g., EEG, MEG) should use white noise burst as US. To the best of our knowledge, this is the first study to compare different US types for fear learning protocols with many trials. Future studies will also be necessary to investigate which US type is superior in detecting influences of interindividual differences on fear learning (e.g., trait anxiety), whether both US types elicit a qualitatively similar fear response, and the suitability of white noise burst in special environments like fMRI or PET.

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Supporting Information

Additional supporting information may be found in the online version of this article:

Table S1: Difficulty values and discrimination values of items in the self-constructed US questionnaire.

4.2 Manuscript 2:
Aversive Imagery Causes De Novo Fear Conditioning

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Aversive Imagery Causes De Novo Fear Conditioning

Erik M. Mueller, Matthias F. J. Sperl, and Christian Panitz
Department of Psychology, University of Marburg

Abstract

In classical fear conditioning, neutral conditioned stimuli that have been paired with aversive physical unconditioned stimuli eventually trigger fear responses. Here, we tested whether aversive mental images systematically paired with a conditioned stimulus also cause de novo fear learning in the absence of any external aversive stimulation. In two experiments ($N = 45$ and $N = 41$), participants were first trained to produce aversive, neutral, or no imagery in response to three different visual-imagery cues. In a subsequent imagery-based differential-conditioning paradigm, each of the three cues systematically coterminated with one of three different neutral faces. Although the face that was paired with the aversive-imagery cue was never paired with aversive external stimuli or threat-related instructions, participants rated it as more arousing, unpleasant, and threatening and displayed relative fear bradycardia and fear-potentiated startle. These results could be relevant for the development of fear and related disorders without trauma.

Keywords

fear, fear conditioning, imagery, aversive conditioning, eyeblink reflex, open data, open materials

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Classical fear conditioning provides a powerful model to explain the acquisition of fear in humans and nonhuman animals (LeDoux, 2014) and is often used to explain the development of anxiety disorders (Lissek et al., 2005; Mineka & Oehlberg, 2008). For example, in this framework, the emergence of a dog phobia could be explained as a consequence of being bitten by a dog; the dog would be the conditioned stimulus (CS), and the bite would be the aversive unconditioned stimulus (US) that becomes associated with the CS. A critical issue with this model, however, is that many patients with anxiety disorders do not recall such experiences (e.g., an aversive US such as a dog bite) in their past (Murray & Foote, 1979; Rachman, 1977), and this raises the question of whether an aversive US must physically occur in order for fear learning to occur.

Observational or vicarious fear-conditioning studies suggest that merely observing someone else receiving an aversive stimulation after CS presentation rather than experiencing the aversive stimulation oneself may suffice for fear learning to occur (Mineka, Davidson, Cook, & Keir, 1984), possibly because of overlapping neural representations of observing someone else in pain and experiencing pain oneself (Morrison, Lloyd, Di Pellegrino, & Roberts, 2004; Olsson & Phelps, 2007). Given that the neural representation of pain may also be activated by merely imagining a painful stimulation (Fairhurst, Fairhurst, Berna, & Tracey, 2012; Jackson, Brunet, Meltzoff, & Decety, 2006; Ogino et al., 2007), associative fear learning could also be based on mental images of the US (King, 1973; Lewis, O'Reilly, Khuu, & Pearson, 2013) and could even occur in the total absence of any physical or observed aversive stimulation. If this is the case, stimulus-contingent aversive imagery could provide an explanation for how fear may develop without aversive in vivo experiences and thus be of high relevance for understanding and treating anxiety disorders.

Previous studies that have investigated the role of an imagined US in conditioning (for a review, see Dadds, Bovbjerg, Redd, & Cutmore, 1997) either provided explicit instructions on CS–US contingencies (e.g., as

Corresponding Author:
Erik M. Mueller, University of Marburg, Department of Psychology, Gutenbergstrasse 18, 35032 Marburg, Germany
E-mail: erik.mueller@staff.uni-marburg.de

used by Arabian, 1982; Soeter & Kindt, 2012) or first conditioned the CS with the physical US and then modulated an existing CS–US association with further US imagery (Jones & Davey, 1990). An open question is whether mental images of an aversive US may cause de novo fear conditioning in the total absence of any physically aversive stimulation, explicit instructions, or previously established CS–US associations. Translated to everyday life, can a person who was never bitten by a dog (and who neither observed how someone else was bitten nor was informed that dogs may bite) develop dog phobia, only because of aversive imagery when seeing a dog?

To investigate this hypothesis, we trained participants to produce specific mental images at the presentation of particular imagery cues. In a subsequent differential fear-conditioning procedure, we systematically paired CSs with these imagery cues but not with an actual US. Two different positive CSs (CSs+) were presented to disentangle CS responses related to aversive imagery from CS responses related to imagery per se. One CS+ was paired with a cue for aversive imagery (aversive CS+) and the other CS+ was paired with a cue for neutral imagery (neutral CS+). In addition, a negative CS (CS–) was presented and paired with an irrelevant stimulus that was physically similar to the imagery cues but was not supposed to prompt any imagery. After an acquisition phase, participants underwent an extinction phase in which the CSs were presented without the respective cues, to further investigate whether imagery-based conditioned fear is extinguished in the same manner as conditioned fear with a physical US (Dadds et al., 1997).

Study 1: Conditioning With an Imagined Thumbtack

Method

Participants and procedure. A total of 45 individuals (age: $M = 22.67$ years, $SD = 2.5$; 36 female, 9 male) with normal or corrected-to-normal vision and without neurological, cardiovascular, or psychiatric conditions participated in this study for course credit. Although we observed large effects for CS+ versus CS– differences in earlier studies with highly potent physically aversive USs (Sperl, Panitz, Hermann, & Mueller, 2016), we expected smaller (medium-size) effects with mental images of aversive events as USs. Under the assumption of medium correlations between measurements ($r = .3$), an alpha error probability of .05, and a power of .8, we used G*Power (Faul, Erdfelder, Lang, & Buchner, 2007) to calculate that a total sample size of 37 would be required to achieve a medium effect size (η_p^2) of .06 for between-conditions differences. A sample size of 45 would allow for a potential data loss of up to 20%.

Participants signed informed consent, filled out a battery of questionnaires to test hypotheses unrelated to the current study, and completed a brief interview, after which they had electrodes attached for recording of the electrocardiogram (ECG) and electrodermal activity (EDA). Afterward, they were seated for a 5-min resting phase. Following an imagery training (see below), participants underwent the imagery-based fear-conditioning paradigm (Fig. 1). At the end, electrodes were detached, a postexperimental interview was conducted, and participants were debriefed and compensated for participation. The study was approved by the local ethics committee of the University of Marburg Psychology Department.

CSs and imagery cues. Three different faces with a neutral expression served as the aversive CS+, neutral CS+, and CS–, respectively (faces obtained from the Ekman faces series; Ekman & Friesen, 1976). The particular CS type of each face was counterbalanced across participants. Three different geometric shapes (a red square, a blue ellipse, and a yellow hexagon) served as imagery cues (aversive cue, neutral cue, no-image cue); assignment of shape and cue type was counterbalanced.

Imagery scripts. Prior to the current study, an online survey with 29 individuals had been conducted to determine a scenario that was considered highly aversive and could be vividly imagined by most individuals. From 10 different scenarios, participants found the "thumbtack-in-the-heel" scenario (see below) to be both highly aversive ($M = 4.24$, $SD = 0.79$, on a scale from 1 to 5) and vividly imaginable ($M = 4.59$, $SD = 0.57$, on a scale from 1 to 5). The "stepping-on-a-coin" scenario was chosen as the nonaversive control scenario because it also provides phasic tactile stimulation of the foot with a metallic object. Imagery scripts were created following the recommendations of Lang (1979) and included a response component to increase the vividness of the imagery (e.g., "your muscles cramp due to the pain of the thumbtack").

The script for the thumbtack-in-the-heel scenario was as follows:

> Imagine the following situation: You walk barefoot through a room, and your right foot steps on a thumbtack. You can feel the thin needle sinking into your heel as you step on the pin with your entire weight. The pain is piercing and intense and spreads from your heel into your leg. Every [red square/blue ellipse/yellow hexagon] that appears on the screen evokes the feeling of the needle pushing into your heel and the piercing and intense pain going through your body. The stinging pain is extremely unpleasant and barely tolerable. Focus on the pain you are experiencing.

You can feel how it spreads from your right heel and you are cramping. You do not want to experience the stinging pain again. With every [red square/blue ellipse/yellow hexagon], you feel the thumbtack pushing into your heel.

The script for the stepping-on-a-coin scenario was as follows:

Imagine the following situation: You walk barefoot through a room, and your right foot steps on a 1-cent coin. You can feel the round metal under your heel when you step on it. The coin feels cool but it is not unpleasant. Every [red square/blue ellipse/yellow hexagon] that appears on the screen evokes the feeling of the round, cool coin under your heel. The contact is not unpleasant and is easily tolerable. Focus on the contact; you are relaxed. With every [red square/blue ellipse/yellow hexagon] that appears on the screen, you feel the round, cool coin under your heel.

The script for the control cue was as follows:

Whenever this [red square/blue ellipse/yellow hexagon] appears on the screen, you do not have to imagine anything. Just sit in your chair, observe the [red square/blue ellipse/yellow hexagon], and think of nothing in particular.

Imagery training. The imagery training was completed prior to the imagery-based-conditioning procedure and started with an auditory recording of the imagery scripts (recordings in German are available at https://doi.org/10.5281/zenodo.2591593). After the auditory instructions were given, participants were reminded two times about each cue–scenario association by instructions on the screen. If necessary, this reminder was repeated until participants were able to report the correct associations.

Imagery-based-conditioning paradigm. The imagery-based-conditioning paradigm consisted of an initial habituation phase, a subsequent acquisition phase, and a

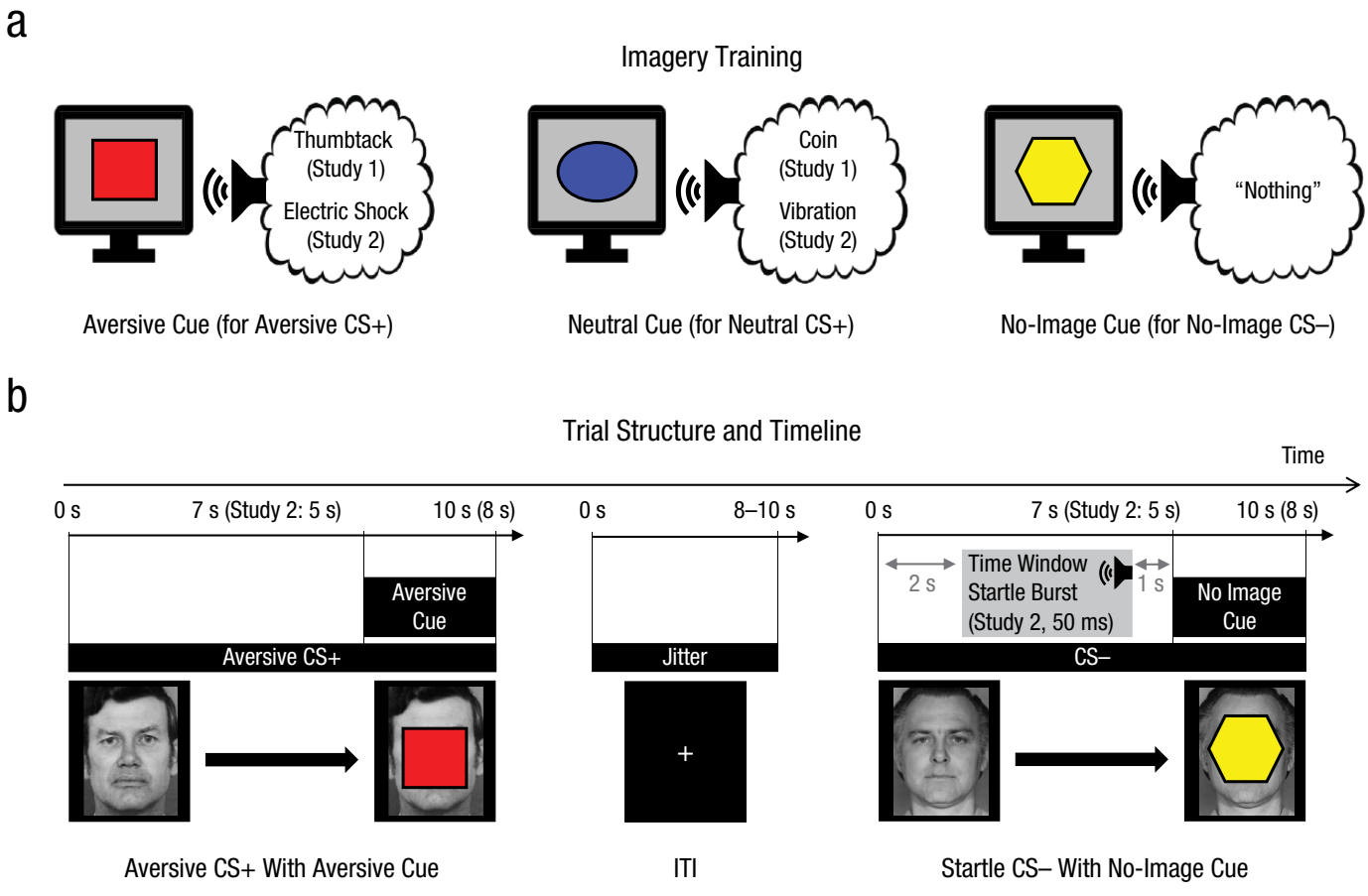

Fig. 1. *(continued on next page)*

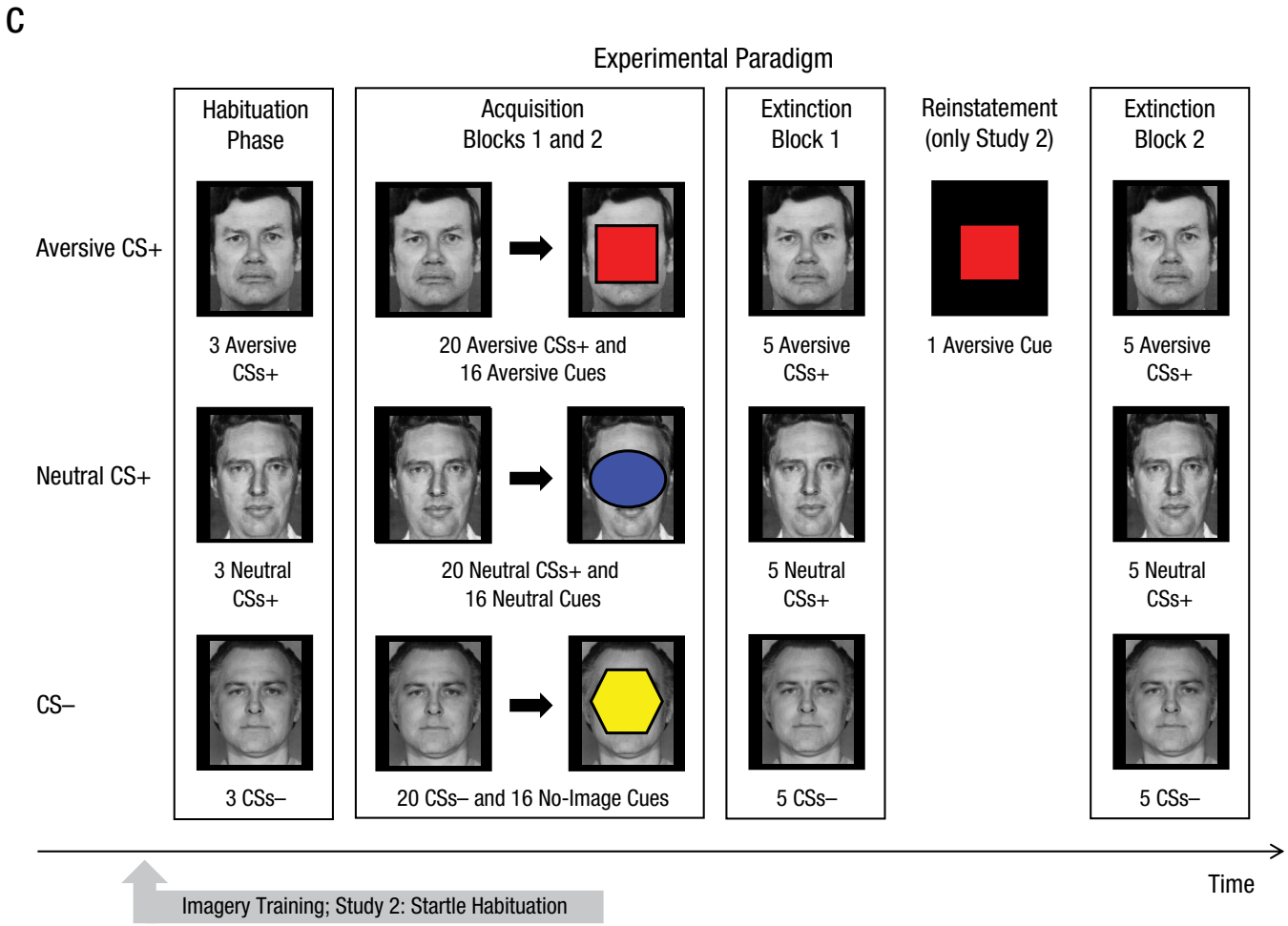

Fig. 1. Schematic depiction of the experimental protocol used in both studies. During imagery training (a), participants were informed of the association between cues (e.g., square, ellipse, hexagon) and imagery scenarios of aversive valence (e.g., stepping on a thumbtack) or neutral valence (e.g., stepping on a coin). In a third condition, participants were instructed not to imagine anything. In Study 2, a circle and a triangle were used instead of the ellipse and hexagon cues, respectively. During the imagery-based differential-conditioning procedure (b), each of three neutral faces (aversive conditioned stimulus, or CS+, neutral CS+, and CS–) was paired with the corresponding cue (aversive cue, neutral cue, no-image cue). All conditioned stimuli (CSs) were presented for 10 s (Study 1) or 8 s (Study 2). CS presentations coterminated with the imagery cue centrally superimposed on the CS for the last 3 s in 80% of the trials. In Study 2, acoustic startle probes were presented during 50% of CS presentations (potential window: 2–4 s after CS onset, i.e., prior to the onset of the imagery cue) and during six intertrial intervals (ITIs). The number and stimuli types used during the habituation, acquisition, and extinction phases are shown in (c). The extinction phase was identical to the acquisition phase, except that the imagery cues were never shown. In Study 2, participants saw the aversive-imagery cue once between two extinction blocks (reinstatement cue).

final extinction phase. The habituation phase consisted of three presentations of each CS for 10 s (intertrial interval, or ITI, jittered from 8 to 10 s) in random order. During each of two sequential acquisition blocks, every CS was presented 10 times for 10 s each, again with a jittered ITI of 8 to 10 s. Of the 10 CS presentations, 8 coterminated with the imagery cue centrally superimposed on the CS for the last 3 s (80% reinforcement). The two extinction blocks were identical to the acquisition blocks, except that the imagery cues were never shown.

Ratings. Before and after each phase and block, participants rated the valence and arousal of each of the three CSs on a 5-point Likert-type scale. In addition, participants reported their subjective experience of fear, anger, and disgust when looking at the CSs. Furthermore, participants were shown the three cues and asked to indicate whether they associated an image with each cue and, if so, to rate the unpleasantness of that image (from 0, *not unpleasant at all*, to 10, *extremely unpleasant*). Participants could also report having no image associated with a cue; these responses, which occurred exclusively to the no-image cue, were coded as 0 for statistical analyses.

Psychophysiological-data recording and reduction. The ECG and EDA were recorded with a BioSemi ActiveTwo system (BioSemi, Amsterdam, The Netherlands) with the

Common Mode Sense and the Driven Right Leg electrodes attached to the right leg (sampling rate = 1024 Hz). For ECG measurement, Ag/AgCl electrodes (4-mm diameter) were applied in a lead-two configuration. In BrainVision Analyzer 2 (Brain Products, Gilching, Germany), the ECG was band-pass filtered (−3 dB at 1 Hz and 30 Hz, fourth-order two-way Butterworth filter, 24 dB/octave roll-off), and R spikes were detected automatically with the EKG Markers solution in the Analyzer software. R spikes were corrected manually if necessary, and nonusable data (e.g., premature systoles, excessive movement artifacts) were removed. Using custom-made MATLAB scripts (MATLAB Version 9.2; The MathWorks, Natick, MA), we then converted the ECG to a time course of interbeat intervals (IBIs), in which the value at each time point reflected the latency between the preceding and the next R spike (Mueller, Stemmler, Hennig, & Wacker, 2013). The IBI time series was then segmented into epochs ranging from −1,000 to 7,000 ms relative to CS onset (CS-evoked IBI) or from −1,000 to 10,000 ms relative to cue onset (cue-evoked IBI), baseline-corrected relative to −1,000 to 0 ms, downsampled to 2 Hz, and averaged across all trials by block and condition.

Heart rate responses to a CS during fear acquisition typically showed a triphasic response pattern (Lipp, 2007) consisting of an initial deceleration (D1), a transient acceleration (A1), and a second deceleration (D2). For analysis of CS-evoked IBIs, the maximum values were extracted for the time periods from 0 ms to 2,000 ms (D1) and 5,000 ms to 7,000 ms (D2), and the minimum values were extracted from 2,000 ms to 5,000 ms (A1). To remove the influence of the preceding components, we then computed peak-to-peak values, for example, the value for A1 was referenced to D1 (corrected A1 = A1 − D1), and the value for D2 was referenced to A1 (corrected D2 = D2 − A1). In addition to analyzing the three components separately, we also analyzed the mean IBI for the entire epoch from 0 ms to 7,000 ms (results are provided in the Supplemental Material available online). For cue-evoked IBIs, in which only a biphasic response was observed, the maximum value from 0 ms to 4,000 ms (D1) and the minimum value from 4,000 ms to 10,000 ms (A1) were taken.

EDA was recorded at the thenar and hypothenar of the nondominant hand with two Ag/AgCl electrodes (5-mm diameter, exosomatic measurement, 1 μA at 16 Hz AC). Electrodes were filled with isotonic (0.5% NaCl) electrolyte medium. Raw EDA was low-pass filtered off-line (1 Hz, same filter specifics as for ECG) and downsampled to 128 Hz. Ledalab 3.4.9 (implemented in MATLAB 9.2) was used for artifact correction and through-to-peak analyses (Benedek & Kaernbach, 2010a, 2010b). All data were visually screened, and technical artifacts were interpolated with spline or cubic interpolation.

Skin conductance responses (SCRs) were defined as the sum of SCR amplitudes of significant SCRs within 1,000 and 5,000 ms after CS or cue onset. SCRs smaller than 0.01 μS were considered zero responses. SCRs were logarithmized, ln(μS + 1), before averaging to obtain a normal distribution. Finally, as in the ECG analysis, SCR through-to-peak scores were averaged within blocks and conditions. Additional, more fine-grained SCR analyses with range correction and exclusion of nonresponders are provided in the Supplemental Material.

Statistical analyses. For analyzing responses to the imagery cues, repeated measures analyses of variance (ANOVAs) with the factors cue type (aversive vs. neutral vs. none) and block of acquisition (first vs. second) were conducted. For analyzing responses to CSs, the repeated measures ANOVAs included the factors CS type (aversive CS+ vs. neutral CS+ vs. CS–) and block of acquisition (first vs. second). Main effects were followed up by pairwise post hoc *t* tests. Greenhouse-Geisser correction was used when applicable. Statistical analyses were performed with SPSS 24.

Results

Responses to imagery cues.

Subjective ratings. A Block (after first acquisition vs. after second acquisition) × Cue Type (aversive vs. neutral vs. none) ANOVA on the pleasantness of mental images revealed a main effect of cue type ($p < .001$; see Fig. 2; for further statistics, see Table S1 in the Supplemental Material), indicating that participants rated the image they had after being shown the aversive cue to be significantly more aversive than the image they had in response to the neutral cue. The Block × Cue Type interaction was not significant ($p = .36$), indicating that images remained aversive throughout the course of acquisition.

Physiological responses. The Block × Cue Type ANOVA on the D1 component revealed no main effects or interactions (all ps > .15). The Block × Cue Type ANOVA on the A1 IBI component revealed a main effect of cue type (see Fig. 2). Follow-up analyses indicated that these effects were driven by increased acceleration to the aversive cue versus the neutral cue ($p = .007$) but not between the aversive cue and the no-image cue ($p = .24$) or the neutral cue and the no-image cue ($p = .23$). Similarly, for SCR, the main effect of cue type was significant ($p = .010$). Direct comparisons revealed increased responses to the aversive cue as opposed to the neutral cue during

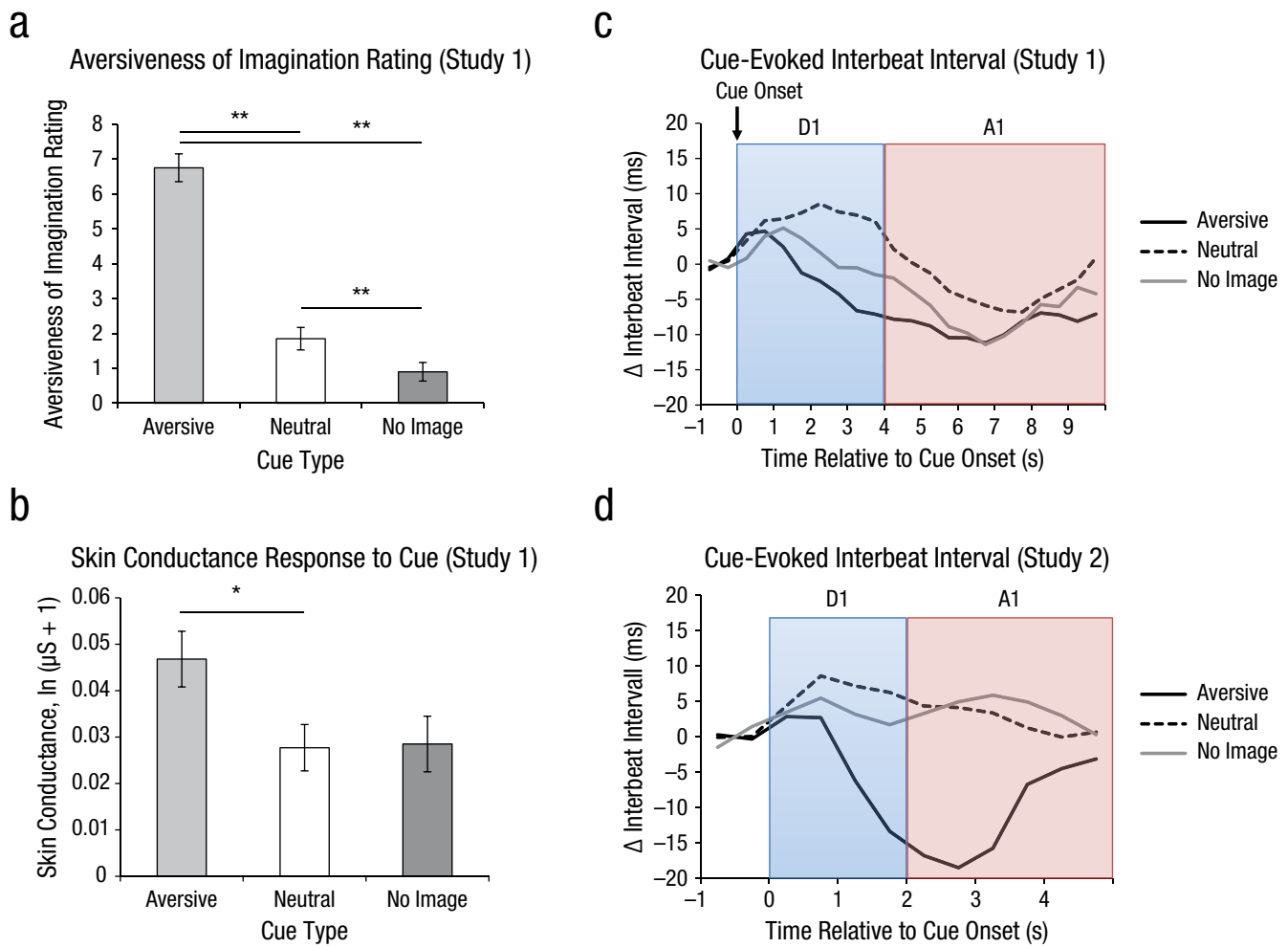

Fig. 2. Responses to imagery cues. The mean unpleasantness rating of mental images in Study 1 is shown in (a) for responses to each of the three cue types. Ratings were made on an 11-point Likert-type scale ranging from 0 (*not at all*) to 10 (*extremely unpleasant*). Mean skin conductance response to each cue type in Study 1 is shown in (b). Error bars in (a) and (b) show repeated measures standard errors of the mean (Masson & Loftus, 2003), and asterisks indicate significant differences between cue types (*$p < .05$, **$p < .001$). Mean evoked heart interbeat interval is shown for Study 1 (c) and Study 2 (d), separately for each cue type during the deceleration time window (D1) and the acceleration time window (A1).

the two acquisition blocks ($p = .001$; see Fig. 2), as well as to the aversive cue as opposed to the no-image cue ($p = .029$). There was no difference between the neutral cue and the no-image cue ($p = .878$).

Together, analyses of ratings and physiological responses thus confirmed that the aversive cue evoked imagery that was perceived as highly unpleasant and was accompanied by increased heart rate and SCR.

Responses to CSs.

Subjective ratings. ANOVAs on self-rated fear after habituation confirmed that participants rated all faces to be similarly fear evoking prior to conditioning ($ps \geq .441$). Importantly, after the first and second acquisition blocks, however, the Block × CS Type ANOVA on fear ratings revealed a main effect of CS type ($p < .001$; see Table S1). As shown in Figure 3, participants rated faces that had been paired with the thumbtack-image cue (aversive CS+) as significantly more fear evoking than faces that had been paired with the coin-image cue (neutral CS+), $t(44) = 3.36$, $p = .002$, or with the no-image cue (CS–), $t(44) = 3.56$, $p < .001$. Very similar main effects of CS type emerged for the anger, disgust, arousal, and valence ratings (all $ps < .008$; see Fig. 4).

Similar to the ANOVAs during acquisition, results of the Block × CS Type ANOVA on ratings during extinction (i.e., after termination of cue presentation) revealed main effects of CS type for fear, anger, disgust, arousal, and valence ratings (all $ps < .033$), indicating that faces previously paired with aversive images continued to evoke negative feelings even if they were no longer paired with image cues.

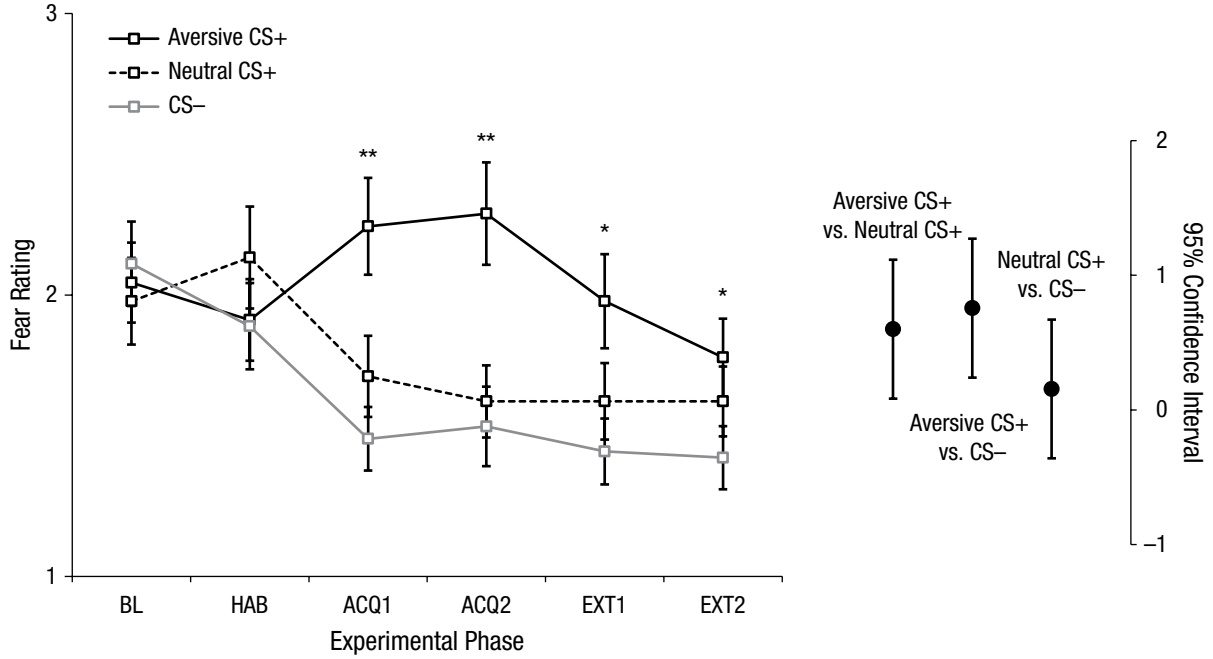

Fig. 3. Fear ratings in Study 1. The graph on the left shows participants' mean ratings of experienced fear when viewing each conditioned stimulus (CS) type during baseline (BL), habituation (HAB), the first acquisition block (ACQ1), the second acquisition block (ACQ2), the first extinction block (EXT1), and the second extinction block (EXT2). Ratings were made on a 5-point Likert-type scale ranging from 1 (*not fearful*) to 5 (*very fearful*). Error bars show repeated measures standard errors of the mean (Masson & Loftus, 2003), and asterisks indicate significant differences between CS types (*$p < .05$, **$p < .001$). On the right, 95% confidence intervals (CIs) are shown for the between-conditions differences during acquisition (collapsed across ACQ1 and ACQ2; Cumming, 2014).

To test whether extinction reduced negative feelings relative to acquisition, we additionally performed a Phase × CS Type ANOVA in which the factor phase consisted of the last block of acquisition versus the last block of extinction. This ANOVA yielded a significant Phase × CS Type interaction for fear ($p = .008$) and arousal ($p = .029$) ratings; the effect of CS type (i.e., the differential fear response) decreased from acquisition toward the end of extinction (fear: η_p^2s = .16 vs. .05; arousal: η_p^2s = .23 vs. .08). There were no significant interactions for valence, anger, or disgust ratings ($ps \geq .155$).

Physiological responses. During habituation, the D1 component did not differ as a function of CS type ($p = .48$). During acquisition, the Block × CS Type ANOVA on the D1 component revealed an interaction of block and CS type ($p = .023$). ANOVAs within blocks revealed that during the beginning of acquisition, IBI did not differ between CS types ($p = .559$), whereas CS type modulated IBI in the second block of acquisition ($p = .023$), as shown in Figure 5. As expected (Notterman, Schoenfeld, & Bersh, 1952; Panitz, Hermann, & Mueller, 2015; Sperl et al., 2016), direct comparisons indicated stronger deceleration for the aversive CS+ than the neutral CS+ (14 vs. 4 ms; $p = .016$). In addition, there was stronger deceleration for the CS– than the neutral CS+ ($p = .035$). The Block × CS Type ANOVA on the other IBI components revealed no significant main effects or interactions involving CS type (all $ps > .5$).

Consistent with successful extinction, the Block × CS Type ANOVA on D1 during extinction did not reveal any main effects or interactions ($ps > .6$). The Block × CS Type ANOVAs on the other IBI components and on EDA revealed no significant main effects or interactions involving CS type during habituation ($ps > .07$), acquisition ($ps \geq .5$), or extinction ($ps \geq .09$).

Study 2: Conditioning With an Imagined Electric Shock

The first study showed that, when contingently paired with aversive mental images, CSs elicit fear responses at the subjective and cardiovascular levels. The aim of Study 2 was to determine whether imagery-based fear conditioning would also work with shorter CS durations and a US that is more typical for classical fear-conditioning studies (i.e., imagery of an electric shock). Moreover, to rule out demand effects, fear-potentiated startle, which is a physiological marker outside of conscious control

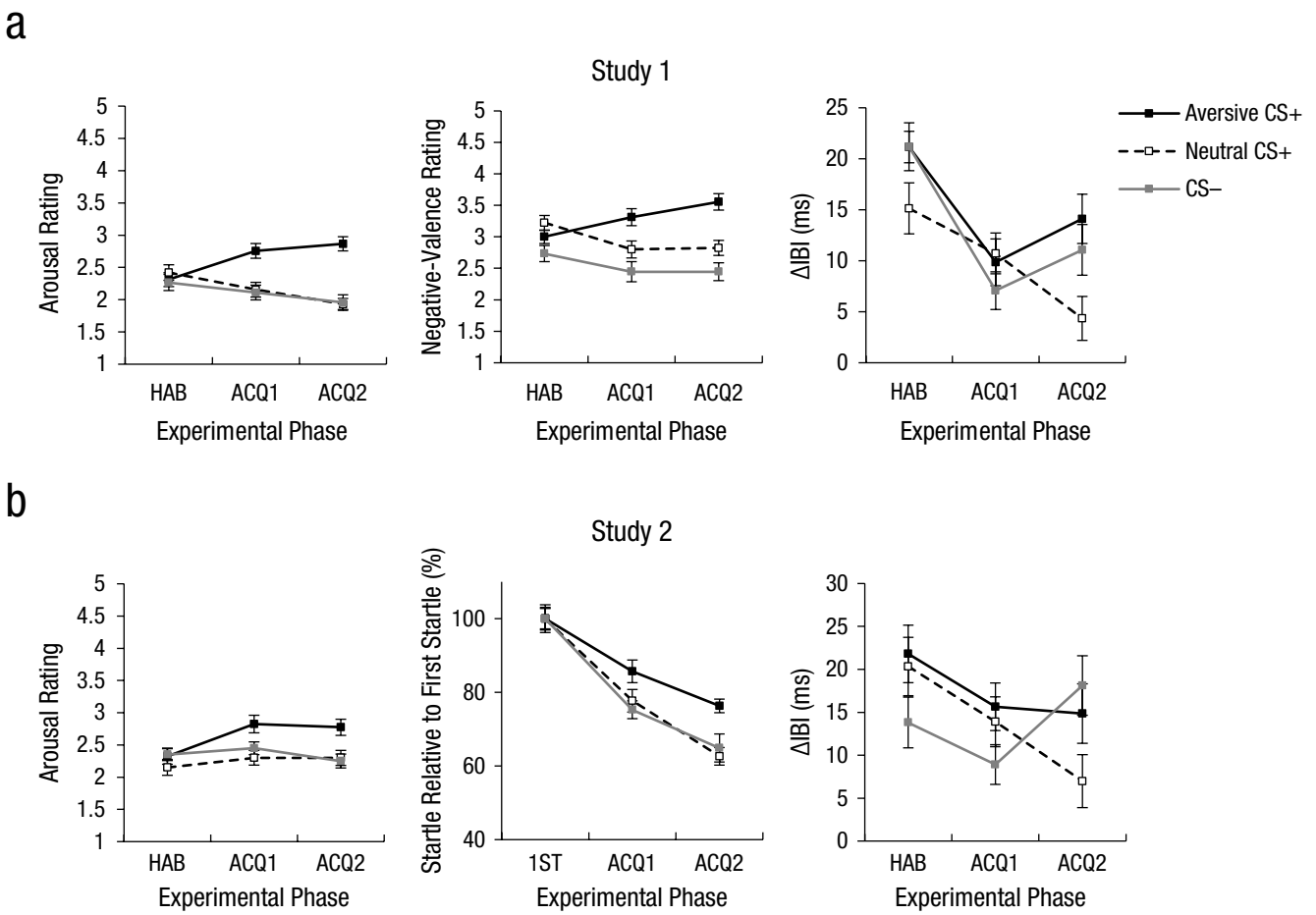

Fig. 4. Responses to conditioned stimuli (CSs). Mean arousal rating, negative-valence rating, and CS-evoked interbeat interval (IBI) in Study 1 are shown in the top row, and mean arousal rating, startle response, and CS-evoked IBI in Study 2 are shown in the bottom row. Startle response was normalized relative to the first startle response during acquisition. In all graphs, results are shown for each CS type during three phases: habituation (HAB), first acquisition block (ACQ1), and second acquisition block (ACQ2). CS-evoked IBIs are shown only for the deceleration time window (D1). Error bars show repeated measures standard errors of the mean (Masson & Loftus, 2003).

(Hamm & Weike, 2005; Lipp, 2007), was assessed in Study 2. Finally, to further explore similarities between imagery-based fear conditioning and fear conditioning based on physical USs, we tested whether imagery of the US after extinction triggers a return of fear (i.e., reinstatement) because it is commonly observed after physical US presentations (Hermans et al., 2005).

Method

Participants and procedure. In Study 2, 41 individuals (age: $M = 23.76$ years, $SD = 2.6$; 29 female, 12 male) participated for course credit. This sample size allowed us to test the central hypotheses with a power of .8, an alpha error probability of .05, and a drop-out rate of 10%. The study was approved by the local ethics committee of the University of Marburg Psychology Department. The overall procedure was identical to that in Study 1, and participants again filled out a battery of questionnaires to test hypotheses unrelated to the current report.

Imagery scripts. In contrast to participants in Study 1, participants in Study 2 were instructed to imagine receiving a strong electric shock on the forearm (aversive imagery) or receiving a mild vibration on the forearm (neutral imagery). The script for feeling a painful electric shock was as follows:

> Imagine the following situation: You sit in a chair; your hands are on the arm rests. An electrode is attached to your left wrist. The electrode provides a short but powerful electric shock whenever a [red square/blue triangle/yellow circle] appears on the screen. The shock spreads throughout your whole body. Every time the [red square/blue triangle/yellow circle] appears on the screen, you

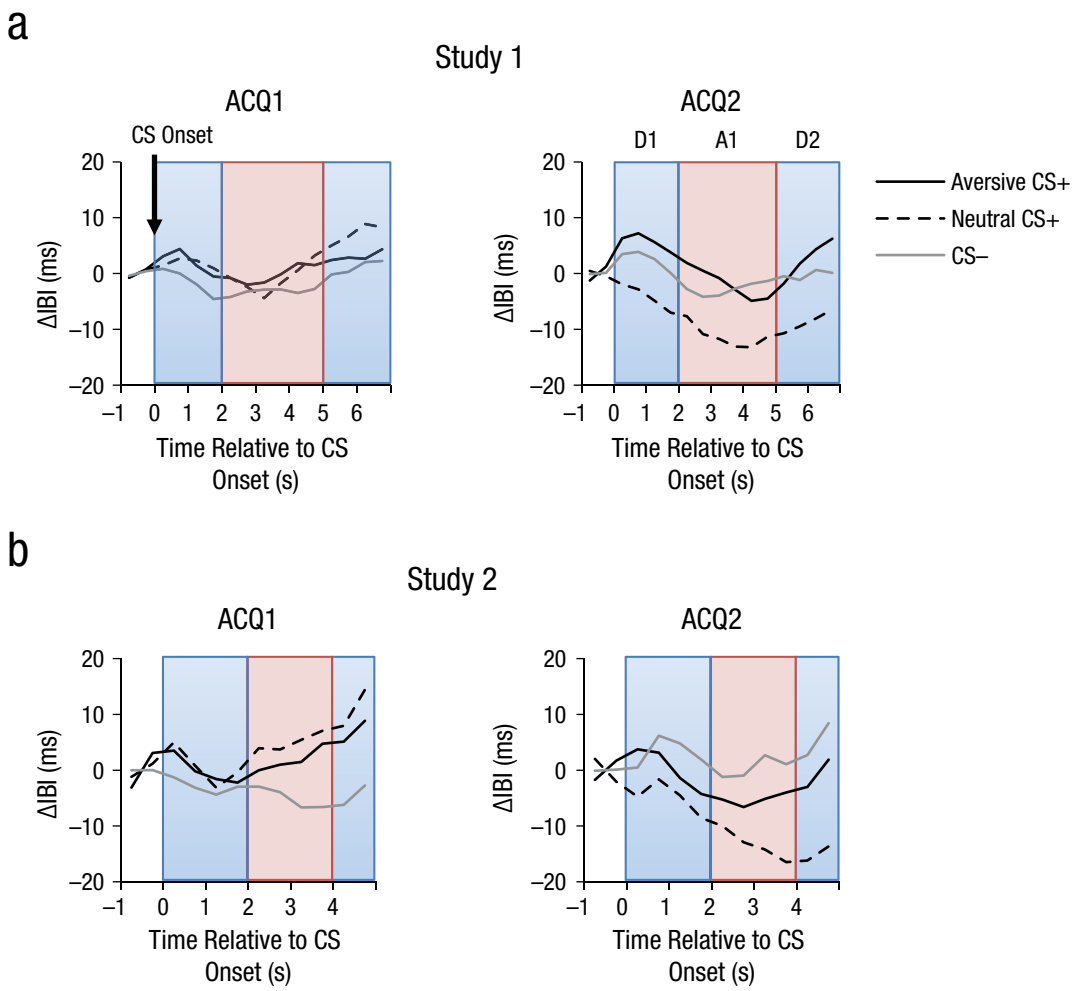

Fig. 5. Mean evoked interbeat interval (IBI) in Study 1 (a) and Study 2 (b) in response to each of the three conditioned stimulus (CS) types during the deceleration time windows (D1 and D2) and the acceleration time window (A1). Results are shown separately for the first acquisition block (ACQ1) and second acquisition block (ACQ2).

receive a painful electric shock. The pain is extremely uncomfortable and barely tolerable. Focus on the pain you are experiencing. You can feel how it spreads throughout your entire body and how your muscles are cramping. You do not wish to experience this pain again. With every [red square/blue triangle/yellow circle], you experience the electric shock again.

The script for feeling a vibration was as follows:

Imagine the following situation: You sit in a chair; your hands are on the arm rests. A wristband is attached to your left wrist. The wristband provides a short vibration whenever a [red square/blue triangle/yellow circle] appears on the screen. Every time the [red square/blue triangle/yellow circle] appears on the screen, you experience this vibration. You can feel the vibration spread throughout your whole body. The sensation is not at all uncomfortable and is easily tolerable. Focus on the vibration you are experiencing. You can feel how it spreads throughout your entire body, and your muscles are relaxed. With every [red square/blue triangle/yellow circle], you experience the vibration again.

The script for the control cue was as follows:

Whenever this [red square/blue triangle/yellow circle] appears on the screen, you do not have to imagine anything. Just sit in your chair, observe the [red square/blue triangle/yellow circle], and think of nothing in particular.

Original imagery scripts in the German language are available at https://doi.org/10.5281/zenodo.2591593.

Imagery-based-conditioning paradigm. The imagery-based-conditioning paradigm was identical to that in Study 1 with the following exceptions. First, the CS was presented for 8 s instead of 10 s. Second, participants saw the aversive-imagery cue once after the first extinction block (reinstatement cue). Third, a circle and a triangle were used instead of the ellipse and hexagon cues, respectively. The ITI, reinforcement rate, cue presentation time, and number of CS presentations were identical to those in Study 1.

Dependent variables.

Ratings. As the effects of conditioning on different affect-rating scales were largely redundant in Study 1, we collected only CS-associated arousal and valence in Study 2. CS ratings (i.e., arousal and valence) from only 40 participants were analyzed because 1 participant claimed after the experiment to have misunderstood the questions. The assessment and analysis were identical to those in Study 1.

Physiological responses. Procedures for SCR and ECG recording and analysis were largely identical to those in Study 1. However, because of the shorter CS presentation latency, only the ECG recording from −1,000 ms to 5,000 ms relative to CS and cue onset were analyzed. Accordingly, the CS-evoked IBI included a D1 component from 0 ms to 2,000 ms, an A1 component from 2,000 ms to 5,000 ms, and a D2 component from 4,000 ms to 5,000 ms. The cue-evoked IBI included a D1 component that was measured as the maximum IBI from 0 ms to 2,000 ms and an A1 component that was measured as the minimum IBI from 2,000 ms to 5,000 ms. To remove the influence of the preceding components in the CS-evoked IBI, we then referenced the value for A1 to D1 (corrected A1 = A1 − D1) and the value for D2 was referenced to A1 (corrected D2 = D2 − A1). Only trials not containing startle probes (see the next section) were used for SCR and IBI analyses. One participant had to be excluded from SCR analyses because of missing data, and 1 participant had to be excluded from IBI analyses for the extinction phase because of excessive artifacts in the ECG recording during that phase.

Fear-potentiated startle. After the resting phase, the startle probe—50 ms duration, 85 dB(A) white-noise burst, 1 ms rise–fall time—was presented five times to allow for an initial startle habituation. In the acquisition and extinction phases, the startle probe was presented during five presentations of each CS in each block (potential window: 2–4 s after CS) and during six ITIs in each block (between 2 s into the ITI and 1 s before its end). Electromyography (EMG) was measured below the left eye on the musculus orbicularis oculi using two Ag/AgCl electrodes (4-mm diameter) and analyzed according to recommendations from Blumenthal et al. (2005). It was first band-pass filtered from 28 Hz to 500 Hz, rectified and low-pass filtered with a time constant of 10 ms, segmented from −50 ms to 250 ms relative to startle onset, and then baseline-corrected from −50 ms to 0 ms.

Because of the good signal-to-noise ratio of startle responses, data were not aggregated within blocks but instead analyzed at the single-trial level (Sevenster, Beckers, & Kindt, 2013; Soeter & Kindt, 2010, 2012) to allow visualization of the learning dynamics during imagery-based fear conditioning. To this end, the maximum value between 20 ms and 150 ms was assessed for each trial in which a startle response was observed that did not begin earlier than 20 ms after startle onset. Single-trial startle magnitudes were T standardized (M = 50, SD = 10) within each participant using ITI startle magnitudes as the reference distribution. In cases of nonresponse, missing values were interpolated on the basis of the value of the preceding available trial of the same category. If there was no preceding trial in that block and category, the value from the succeeding available trial was taken instead. If there was no trial in one acquisition block and category, the participant was excluded, yielding a final sample for startle analysis of 29 for acquisition and 26 for extinction. Finally, single-trial startle responses were normalized as the percentage of the first startle response during the respective condition. For the statistical analysis of fear-potentiated startle, a CS Type × Trial ANOVA was performed.

Results

Responses to image cues.

Subjective ratings. The Block × Cue Type ANOVA on the unpleasantness ratings of the cue-related image revealed a main effect of cue ($p < .001$), indicating that the image prompted by the aversive cue was rated as more unpleasant than the image prompted by the neutral cue or the no-image cue, whereas there was no difference between ratings for the neutral cue and the no-image cue.

Physiological responses. As in Study 1, the Block × Cue Type ANOVA on the A1 revealed a significant main effect of cue type ($p = .013$), whereas the same ANOVA on the D1 component during acquisition revealed no main effects or interactions (all ps > .3). Mirroring the results of Study 1 (see Fig. 2d), follow-up analyses indicated that these effects were driven by a stronger acceleration component to the aversive cue than to the neutral cue ($p = .022$) and to the aversive cue than to the no-image cue ($p = .015$) but not to the neutral cue than to the no-image cue ($p = .52$). A Block × Cue Type ANOVA on SCR revealed only a main effect of block ($p = .046$), which was due to smaller responses in the second block than the first block, and no other significant main effects

or interactions (all *p*s ≥ .309). As in Study 1, the aversive cue thus evoked a mental image that was perceived as highly unpleasant and was accompanied by increased heart rate. Meanwhile, in this study, increased SCR to the aversive-imagery cue was not observed.

Responses to CSs.

Subjective ratings. At baseline and after habituation, participants rated all faces to be similarly arousing and pleasant (*p*s ≥ .305). The CS Type × Block ANOVA on the arousal ratings revealed a main effect of CS type ($p = .017$; see Fig. 4). Post hoc *t* tests showed increased arousal for the aversive CS+ than for the neutral CS+ ($p = .020$) and the aversive CS+ than the CS– ($p = .021$) but not between the neutral CS+ and the CS– ($p = .742$). With regard to the valence ratings, the ANOVA revealed a significant effect of block ($p = .033$) and a trend for CS type ($p = .074$). In line with the arousal ratings and Study 1, exploratory *t* tests indicated increased unpleasantness of the aversive CS+ compared with the neutral CS+ ($p = .030$). There were no significant differences between ratings for the aversive CS+ and the CS– ($p = .330$) or the neutral CS+ and the CS– ($p = .170$).

During extinction, the Block (before reinstatement vs. after reinstatement) × CS Type ANOVAs on arousal and valence ratings showed a significant main effect of CS type only for the arousal ratings ($p = .004$), which was not further modulated by block ($p = .641$), thus providing no evidence for complete extinction or reinstatement. Similarly, an ANOVA on ratings after the last block of acquisition and the last block of extinction revealed no interactions of block and CS type for the arousal or valence ratings (*p*s > .6). Taken together, the ratings of Study 2 provide no evidence for successful extinction or reinstatement.

Peripheral measures. During habituation, there was no effect of CS type in any of the three cardiac components (*p*s > .32). The Block × CS Type ANOVA on D1 for acquisition revealed a marginally significant interaction of block and CS type ($p = .070$; see Fig. 4), comparable with the significant effect in Study 1. The overall pattern mirrored the results of Study 1, suggesting marginally higher IBI and relative deceleration to the aversive CS+ and CS– compared with the neutral CS+ in the second but not the first block of acquisition (see Fig. 5). As in Study 1, the Block × CS Type ANOVAs on D1 during extinction and the analyses of the other cardiac components and of SCR during any of the three phases revealed no significant main effects or interactions involving CS type (*p*s > .12).

Fear-potentiated startle. The CS Type × Trial ANOVA revealed a main effect of CS type ($p = .037$) and a main effect of trial ($p < .001$). Post hoc *t* tests on the main effect of CS type revealed a significant difference between the aversive CS+ and the CS– ($p = .023$) and (marginally) between the aversive CS+ and the neutral CS+ ($p = .054$) but not between the neutral CS+ and the CS– ($p = .970$).

Trial-wise *t* tests revealed that startle probes during the aversive CS+ evoked enhanced startle responses relative to the CS– in Trial 3 ($p = .043$) of the first acquisition block and in Trial 1 ($p = .036$), Trial 2 ($p = .084$), Trial 3 ($p = .019$), and Trial 4 ($p = .022$) of the second acquisition block and that the aversive CS+ evoked enhanced startle relative to the neutral CS+ in Trial 1 ($p = .035$), Trial 2 ($p = .054$), Trial 3 ($p = .021$), and Trial 4 ($p = .001$) of the second acquisition block (see Fig. 6). Specifically, a dishabituation between the last trial of the first acquisition block (Trial 5) and the first trial of the second acquisition block (Trial 6, following a short break) was observed for the aversive CS+ (from 76% to approximately 81% of the first startle response), whereas the response during the other two CSs remained at about 68%.

The CS Type × Trial ANOVA for the startle responses during extinction revealed only main effects of trial ($p < .001$) but no other significant main effects or interactions (*p*s ≥ .42). Trial-wise *t* tests for extinction revealed no enhanced startle responses for the aversive CS+ relative to the neutral CS+ (*p*s ≥ .073) or CS– (*p*s ≥ .169).

Discussion

The goal of the current research was to test whether fear can be conditioned de novo with aversive mental images as USs only. To this end, we conducted two studies in which different neutral face photographs were contingently paired with specific cues that had been previously trained to prompt aversive, neutral, or no imagery in 41 and 45 participants, respectively. Across studies, participants rated neutral faces as more fear evoking, unpleasant, and arousing, and they responded with relative cardiac deceleration and fear-potentiated startle if the faces had been paired with aversive imagery compared with neutral or no imagery. Because these findings indicate that associative fear learning may occur in the total absence of aversive physical stimulation, vicarious experiences, or explicit instructions, our results are relevant for understanding how phobias and anxiety disorders may develop in the absence of prior physically aversive experiences.

Most importantly, CS ratings revealed that faces, which were initially perceived as neutral, were later rated as more unpleasant, arousing, and fear evoking if they had been paired with cues for aversive as opposed to neutral or no imagery. It can be assumed that these cues prompted participants to produce the

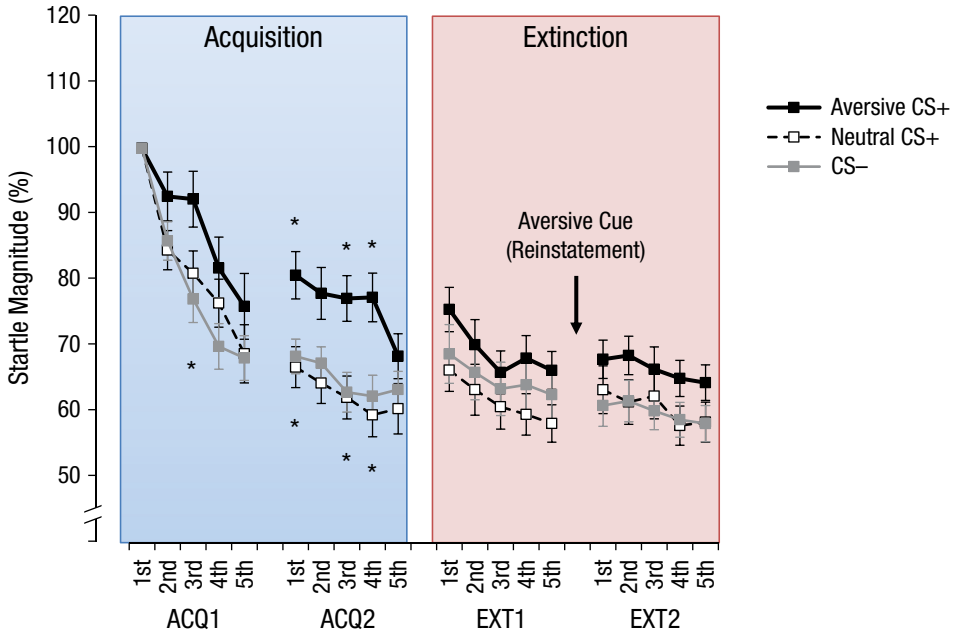

Fig. 6. Mean normalized single-trial eyelid startle responses to the 85-dB noise burst as a function of the conditioned stimulus (CS) type during the first acquisition block (ACQ1), second acquisition block (ACQ2), first extinction block (EXT1), and second extinction block (EXT2). The aversive cue was presented between EXT1 and EXT2 to test for reinstatement of fear. Error bars show repeated measures standard errors of the mean (Masson & Loftus, 2003). Asterisks above the lines indicate significant differences between the aversive CS+ and the neutral CS+, and asterisks below the lines indicate significant differences between the aversive CS+ and the CS– ($p < .05$, two tailed).

intended mental images, given that participants rated the mental images in response to the aversive cue as highly unpleasant and showed cardiac acceleration and increased SCRs to the aversive cue. Because (a) none of the faces had ever been paired with an aversive physical stimulus, (b) no instructions regarding the faces had ever been given, and (c) participants had not observed anyone else receiving an aversive stimulation in response to the faces, the higher arousal, negative valence, and fear ratings to the aversive CS+ than to the neutral CS+ and the CS– in Studies 1 and 2 can be ascribed only to the different mental images prompted by the associated cues.

At the cardiac level, the aversive CS+ evoked more cardiac deceleration compared with the neutral CS+ after the first acquisition block in Study 1 and (marginally significantly) in Study 2. Because fear-conditioned CSs+ generally evoke cardiac deceleration or "fear bradycardia" (Notterman et al., 1952; Panitz et al., 2015; Sperl et al., 2016), this finding further supports successful imagery-based fear learning from Block 1 to Block 2. In addition, the two nonthreatening stimuli differed from each other; there was a relative acceleration to the neutral CS+ as opposed to the CS–, consistent with imagery tasks evoking cardiac acceleration (Vrana & Lang, 1990).

With regard to the eyelid startle magnitude, which is believed to be a relatively pure correlate of stimulus valence in both classical fear conditioning and fear imagery (Hamm & Weike, 2005; Vrana & Lang, 1990), noise bursts given during aversive CS+ evoked stronger startle responses than bursts given during the neutral CS+ or CS–. As with cardiac deceleration, this effect increased throughout the course of learning and was particularly pronounced in the second half of the acquisition phase. At the same time, we did not observe higher electrodermal responses to the CS+ than to the CS– as we have found with physical USs using the same type of CS (Mueller, Panitz, Hermann, & Pizzagalli, 2014; Panitz et al., 2018; Sperl et al., 2016). Furthermore, in Study 2, imagery of a US did not trigger a reinstatement as would be expected with a physical US presentation (Hermans et al., 2005). Together, this suggests that de novo fear acquisition based on imagery mirrors physical-US-based fear conditioning with regard to some factors (i.e., subjective report, fear-potentiated startle, fear bradycardia) but not all factors (i.e., EDA, reinstatement).

An open question is whether the fear conditioning, as observed in both studies, was actually caused by the mental images that were paired with the CSs. Alternatively, the aversive-imagery cues themselves may

have acquired aversive properties during the initial imagery-training procedure and served as a second-order conditioning US (Rizley & Rescorla, 1972). Although this is somewhat speculative at this point, such a mechanism may have far-reaching clinical implications because it would suggest that cues remotely associated with aversive imagery (rather than aversive imagery per se) may cause new fear learning. To probe the involvement of second-order conditioning, researchers may in the future control for cue valence, for example, by collecting cue valence ratings or by applying more indirect approaches to assess stimulus valence. Alternatively, researchers may include a control group that receives the initial imagery training but is instructed to not engage in imagining when cues are presented during conditioning.

Furthermore, the observed fear responses to the aversive CS+ may not have been caused by the actual imagery of an aversive event but may instead relate to propositional knowledge. Although this is a general issue of human associative-learning studies (Mitchell, De Houwer, & Lovibond, 2009), the startle potentiation during the aversive CS+ compared with both the neutral CS+ and CS– in Study 2 shows that associative learning could also be observed with regard to threat responses that are largely outside of cognitive control (Hamm & Weike, 2005; Lipp, 2007). Moreover, the observed relative fear bradycardia to the aversive CS+ in Studies 1 and 2 supports the notion that the aversive CS+ indeed triggered fear responses across multiple response systems, suggesting that the acquired association of the aversive CS+ and aversive imagery goes beyond merely propositional knowledge.

The type of learning that is captured with this novel paradigm is potentially relevant for anxiety disorders and other aspects of human functioning, particularly in light of the relevance of imagery for mental disorders and their treatment (Pearson, Naselaris, Holmes, & Kosslyn, 2015). This type of imagery-based learning connects truly existing external stimuli to threatening images or, by extension, reality to fantasy. With such connections, the emergence of dog phobia does not require being bitten by a dog but it would suffice to merely imagine being bitten when encountering dogs. Similarly, imagery of social embarrassment, suffocation, back pain, or even terrorist attacks may be highly relevant for the emergence and treatment of social phobia, agoraphobia, pain disorder, and social prejudice, when contingently paired with seeing other individuals, subway trains, movements, or foreigners, respectively.

It should be noted that the content and time course of experimentally induced imagery cannot be perfectly controlled. After contingencies are learned, participants may initiate imagery before cues are presented. As a consequence, recordings of conditioned responses after CS presentations may have been confounded with unconditioned responses to the mental images. In contrast to this assumption, however, we observed a dissociation of unconditioned responses and conditioned responses at the cardiovascular level, which is typically found in classical fear-conditioning studies (Lipp, 2007): relative cardiac deceleration to the aversive CS+, but cardiac acceleration to the US or, in the current studies, the aversive cue. Moreover, participants may have visualized an image when US presentations were not intended (e.g., during the CS–, nonreinforced trials, or the extinction phase) or, alternatively, may have avoided unpleasant mental images by not vividly imagining the US or not imagining the US at all. Because such behavior may have led to enhanced or reduced imagery-based conditioning, respectively, the reported effect sizes may not accurately reflect the actual potential of aversive images to induce fear learning in real life.

Taken together, the present studies showed that subjective and physiological fear responses were evoked by neutral faces, which were never paired with any aversive physical stimuli, any observations, or any explicit instructions but only with cues for aversive imagery. When contingently paired with neutral stimuli, particular images may thus lead to de novo conditioning, which is of potential relevance for anxiety disorders, social prejudice, and other dimensions of human functioning.

Action Editor

Ian H. Gotlib served as action editor for this article.

Author Contributions

E. M. Mueller developed the study concept. All the authors contributed to the study design. C. Panitz programmed the experiment. Testing and data collection were performed by students supervised by C. Panitz and M. F. J. Sperl. Data preprocessing was performed by M. F. J. Sperl (electrodermal activity) and C. Panitz (all other physiological variables). E. M. Mueller analyzed and interpreted the data. C. Panitz confirmed the reproducibility of all reported results. E. M. Mueller drafted the manuscript, and C. Panitz and M. F. J. Sperl provided critical revisions. E. M. Mueller and M. F. J. Sperl created the figures. M. F. J. Sperl made the data, analysis scripts, and codebooks publicly available. All the authors approved the final manuscript for submission.

ORCID iD

Matthias F. J. Sperl https://orcid.org/0000-0002-5011-0780

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Declaration of Conflicting Interests

The author(s) declared that there were no conflicts of interest with respect to the authorship or the publication of this article.

Supplemental Material

Additional supporting information can be found at http://journals.sagepub.com/doi/suppl/10.1177/0956797619842261

Open Practices

Deidentified data for both experiments, a codebook, data-analysis scripts, and audio recordings of the imagery scripts have been made publicly available via Zenodo and can be accessed at https://doi.org/10.5281/zenodo.2591593. Face stimuli were part of the Ekman face series (Ekman & Friesen, 1976) and cannot be posted without permission. The stimulus IDs are JJ3-4, EM2-4, and GS1-4. The design and analysis plans for the studies were not preregistered. The complete Open Practices Disclosure for this article can be found at http://journals.sagepub.com/doi/suppl/10.1177/0956797619842261. This article has received the badges for Open Data and Open Materials. More information about the Open Practices badges can be found at http://www.psychologicalscience.org/publications/badges.

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4.3 Manuscript 3:

Learning Dynamics of Electrophysiological Brain Signals During Human Fear Conditioning

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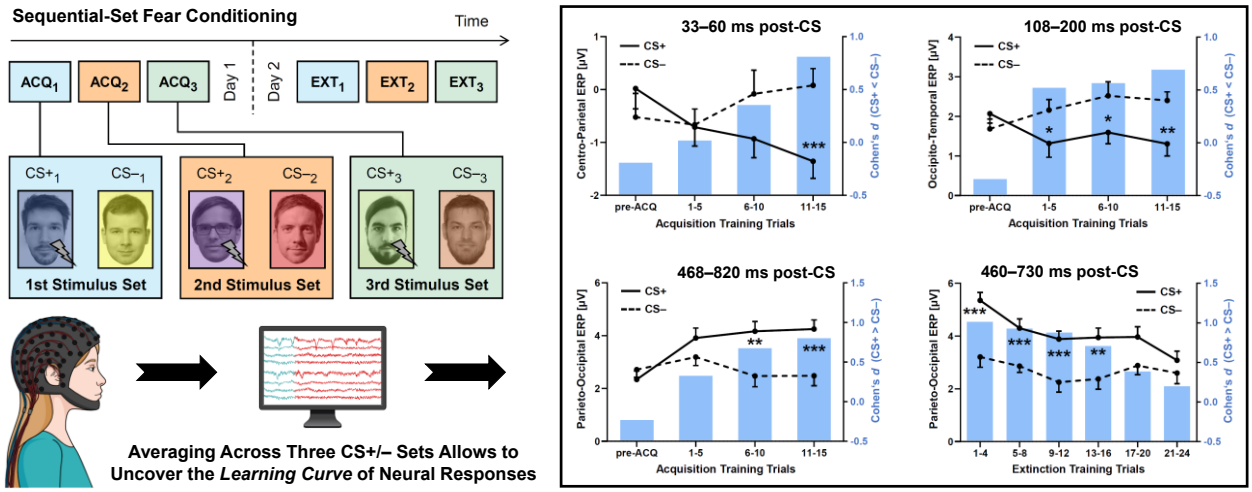
I received an **RTG 2271 Poster Award** for this project at the 2019 retreat of the **Research Training Group (RTG) 2271 "Breaking Expectations"** in Hirschegg, Austria.

Graphical Abstract:



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Learning dynamics of electrophysiological brain signals during human fear conditioning

Matthias F.J. Sperl[a,b,*], Adrian Wroblewski[c], Madeleine Mueller[c,d], Benjamin Straube[c], Erik M. Mueller[a]

[a] Department of Psychology, Personality Psychology and Assessment, University of Marburg, 35032 Marburg, Germany
[b] Department of Psychology, Clinical Psychology and Psychotherapy, University of Giessen, 35394 Giessen, Germany
[c] Department of Psychiatry and Psychotherapy, Translational Neuroimaging Marburg, University of Marburg, 35039 Marburg, Germany
[d] Department of Systems Neuroscience, University Medical Center Hamburg-Eppendorf, 20246 Hamburg, Germany

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ABSTRACT

Electrophysiological studies in rodents allow recording neural activity during threats with high temporal and spatial precision. Although fMRI has helped translate insights about the anatomy of underlying brain circuits to humans, the temporal dynamics of neural fear processes remain opaque and require EEG. To date, studies on electrophysiological brain signals in humans have helped to elucidate underlying perceptual and attentional processes, but have widely ignored how fear memory traces *evolve* over time. The low signal-to-noise ratio of EEG demands aggregations across high numbers of trials, which will wash out transient neurobiological processes that are induced by learning and prone to habituation. Here, our goal was to unravel the plasticity and temporal emergence of EEG responses during fear conditioning. To this end, we developed a new sequential-set fear conditioning paradigm that comprises three successive acquisition and extinction phases, each with a novel CS+/CS- set. Each set consists of two different neutral faces on different background colors which serve as CS+ and CS-, respectively. Thereby, this design provides sufficient trials for EEG analyses while tripling the relative amount of trials that tap into more transient neurobiological processes. Consistent with prior studies on ERP components, data-driven topographic EEG analyses revealed that ERP amplitudes were potentiated during time periods from 33–60 ms, 108–200 ms, and 468–820 ms indicating that fear conditioning prioritizes early sensory processing in the brain, but also facilitates neural responding during later attentional and evaluative stages. Importantly, averaging across the three CS+/CS- sets allowed us to probe the temporal evolution of neural processes: Responses during each of the three time windows gradually increased from early to late fear conditioning, while long-latency (460–730 ms) electrocortical responses diminished throughout fear extinction. Our novel paradigm demonstrates how short-, mid-, and long-latency EEG responses change during fear conditioning and extinction, findings that enlighten the learning curve of neurophysiological responses to threat in humans.

1. Introduction

Rapid learning about threats is essential for survival (LeDoux and Daw, 2018), but it can also contribute to the etiology and maintenance of pathological fear (Parsons and Ressler, 2013). Patients with anxiety disorders exhibit elevated fear conditioning and resist fear extinction (Lissek et al., 2005; Duits et al., 2015). Fear conditioning (LeDoux, 2014) describes a learning procedure during which an initially neutral stimulus (conditioned stimulus, CS) elicits fear after becoming associated with an aversive event (unconditioned stimulus, US). Conversely, when the CS is presented in the absence of the aversive US, the fear response is extinguished and the strength of behavioral fear measures declines (Bouton, 2017). Neurophysiological mechanisms of fear conditioning and extinction have been widely investigated in animals (Tovote et al., 2015), leading to the development of neurobiological models of threat processing (Calhoon and Tye, 2015; McCullough et al., 2016). In animals, recording intracranial electrical activity of single units allows to unravel dynamics of threat processing with high spatial and temporal precision (Fadok et al., 2017).

Translating insights from animal studies on neural threat circuits into the human realm is challenging (Janak and Tye, 2015; Flores et al., 2018; Haaker et al., 2019): Available methods like fMRI or EEG lack either temporal or spatial specificity, respectively (Logothetis et al., 2001; Hajcak et al., 2019). Several studies have used fMRI to reveal

* Corresponding author at: Gutenbergstr. 18, 35032 Marburg, Germany.
E-mail addresses: matthias.sperl@staff.uni-marburg.de (M.F.J. Sperl), adrian.wroblewski@med.uni-marburg.de (A. Wroblewski), mad.mueller@uke.de (M. Mueller), benjamin.straube@staff.uni-marburg.de (B. Straube), erik.mueller@staff.uni-marburg.de (E.M. Mueller).

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the anatomy of fear conditioning in humans, including the amygdala (Greco and Liberzon, 2016; but see Fullana et al., 2016), insula, hippocampus, and prefrontal areas (Fullana et al., 2016). Imaging techniques like fMRI are well suited to study slower brain processes, but the study of fast and transient cortical processes requires techniques with a much higher temporal resolution. Importantly, EEG or MEG offer perfect temporal accuracy to detect changes in brain activity over milliseconds (Miskovic and Keil, 2012). These methods allow to disentangle individual neural mechanisms (Erickson et al., 2018) and to assess whether amplified cortical responses are processed at automatic or rather strategic stages (Klumpp and Shankman, 2018).

Prioritized processing of threat cues can occur at different sensory and cognitive levels (Gupta et al., 2019; Wieser and Keil, 2020), including sharpened tuning of visuocortical neurons (Stegmann et al., 2020). Electromagnetic methods are pivotal tools to investigate how threat can guide perceptual, attentional, and evaluative processing stages (Lang and Bradley, 2010; Bublatzky and Schupp, 2012; Miskovic and Keil, 2012). Some studies suggest that fear conditioning facilitates neural processing at very early stages which begin already at latencies < 40 ms after stimulus onset (Bröckelmann et al., 2011; Kluge et al., 2011; Morel et al., 2012; Steinberg et al., 2013). Others reported heightened parieto-occipital amplitudes to CS+ versus CS- from 60 to 90 ms (Stolarova et al., 2006; Hintze et al., 2014; Thigpen et al., 2017) as indicated by the C1 component amplitude. These results emphasize that short-term plasticity in primary visual neurons may be responsible for biased threat perception already during early latencies. A few studies propose that conditioned responses amplify amplitudes of the P1 (~80–150 ms) and of the face-sensitive N170 (~130–200 ms) components (Pizzagalli et al., 2003; Pourtois et al., 2004; Liu et al., 2012b; Levita et al., 2015; Camfield et al., 2016; Muench et al., 2016), but results are mixed. Specifically, Muench et al. (2016) showed more positive P1 amplitudes at lateral parietal electrode sites for fear-conditioned faces, but only during a self-relevant threat context. Levita et al. (2015) and Camfield et al. (2016) found more negative amplitudes at occipito-temporal channels and interpreted these effects as elevated N170 responses. However, other studies failed to replicate differential fear responses during these mid-latency periods (Stolarova et al., 2006; Seligowski et al., 2018; Stolz et al., 2019), and inconsistent findings (Schindler and Bublatzky, 2020) may arise from increased attention toward both threat (CS+) and safety (CS-) cues (Bublatzky and Schupp, 2012).

Wieser and Keil (2020) argue that modulations of the C1, P1, and N170 amplitudes reflect neural correlates of a somewhat “broad” discrimination between threat and non-threat cues. In contrast, effects on late-latency (> 300 ms) event-related potential (ERP) components are assumed to indicate sustained activation of motivational neural systems (Cuthbert et al., 2000; Hajcak and Foti, 2020), related to widespread perceptual, motivational, and motor signals (Wieser and Keil, 2020). Numerous studies confirmed that the late positive potential (LPP) at parieto-occipital sensors is reliably enhanced to fear-conditioned stimuli (Panitz et al., 2015, 2018; Pastor et al., 2015; Bacigalupo and Luck, 2018; Seligowski et al., 2018; Ferreira de Sá et al., 2019; Pavlov and Kotchoubey, 2019; Stolz et al., 2019). The exact time window for LPP scoring varies between studies, but the majority restricted statistical analyses to the 300–800 ms period.

Taken together, EEG studies have not only reported that fear conditioning modulates rather early (Miskovic and Keil, 2012) ERP components (which can be interpreted as facilitated perception through early visual cortical plasticity), but also found effects on later (Panitz et al., 2015; Bacigalupo and Luck, 2018) ERP components (reflecting sustained engagement with threat). All of these results typically rely on averaging across a massive number of trials to achieve an acceptable signal-to-noise ratio for EEG (Huffmeijer et al., 2014), thereby neglecting any changes in the course of learning that would be expected from theoretical models (Rescorla and Wagner, 1972). Notably, neurophysiological responses to the CS change across trials due to habituation and learning. For example, single-trial analyses suggest that P1 modulations change throughout fear conditioning, depending on involved attention mechanisms (Liu et al., 2012b). These temporal dynamics are often of particular interest and considered in fMRI studies (Yin et al., 2018). When averaging across all EEG trials of an acquisition session, however, any information about the learning dynamics within that session will typically be lost. Furthermore, modulation of transient ERP components can be overlooked due to habituation across many trials.

During the last decade, a growing body of studies has begun to translate electrophysiological signatures of learned fear from the rodent model to humans (e.g., Thigpen et al., 2017; Bacigalupo and Luck, 2018; Seligowski et al., 2018; Roesmann et al., 2020). Although learning may be defined as a *change in neural activity* due to experience (Ferreira de Sá et al., 2019), human electrophysiological studies of fear conditioning have widely been unable to investigate how brain signals to threat stimuli actually *change* over the course of learning. To close this gap and to overcome the methodological challenges described above, we developed a new sequential-set fear conditioning paradigm that comprises three successive acquisition phases, each with a *novel* CS+/CS- set. We validated our new paradigm by means of a data-driven approach to identify differences in EEG topography between experimental conditions and by testing whether fear conditioning effects in one stimulus set are also present across the other two with regard to EEG components, subjective ratings, electrodermal activity, and fear bradycardia. As outlined above, findings on the timing of ERP effects during fear conditioning are heterogeneous, and, for some components (e.g., P1 and N170), results diverge (Pizzagalli et al., 2003; Ferreira de Sá et al., 2019). The majority of studies focused on specific *a priori* selected components, with latencies and electrodes varying across studies. Thereby, this approach makes the selection of parameters a somewhat speculative endeavor, which may result in missing any effects that do not align with *a priori* selected latencies and electrodes. To address this issue, we applied a data-driven approach to identify relevant time windows for ERP analyses. Following previous fear conditioning studies, we were specifically interested in ERP responses within 1000 ms.

Our new sequential-set fear conditioning paradigm allows probing the temporal unfolding of brain mechanisms with EEG, thereby complementing functional anatomical knowledge obtained from fMRI research. On the one hand, averaging across trials from three CS sets ensures a high signal-to-noise ratio. On the other hand, by using three CS sets there are fewer repetitions of a single stimulus. Importantly, our paradigm triples the relative amount of trials that capture habituation-prone neural responses given that *novel* pictures have been shown to lead to a complete recovery of attenuated ERP amplitudes (Codispoti et al., 2006, 2007). While reducing habituation, sequential-set conditioning should therefore ensure a good signal-to-noise ratio for tapping into more transient neurophysiological processes. Specifically, we hypothesized that from early to late fear conditioning trials ERP amplitudes would gradually increase to CS+ versus CS-, particularly within the aforementioned time windows and locations roughly relating to the C1, P1, N170, and LPP components. Conversely, CS+ versus CS- differences in ERP amplitudes should vanish throughout fear extinction.

2. Materials and methods

2.1. Participants

Twenty-four healthy, right-handed, and non-smoking students at the University of Marburg participated in this study. One subject did not complete the study, and two subjects were excluded because of excessive artifacts in EEG data, yielding a total sample of $N = 21$ participants (mean age = 20.76 years, $SD = 2.28$ years, range: 18–26 years; 85% females). Based on our previous studies (e.g., Panitz et al., 2018; Stolz et al., 2019), we expected medium to large effect sizes for conditioned fear responses. Thus, we used G*Power (Faul et al., 2007) to determine the sample size needed for an effect size of Cohen’s $d = 0.7$.

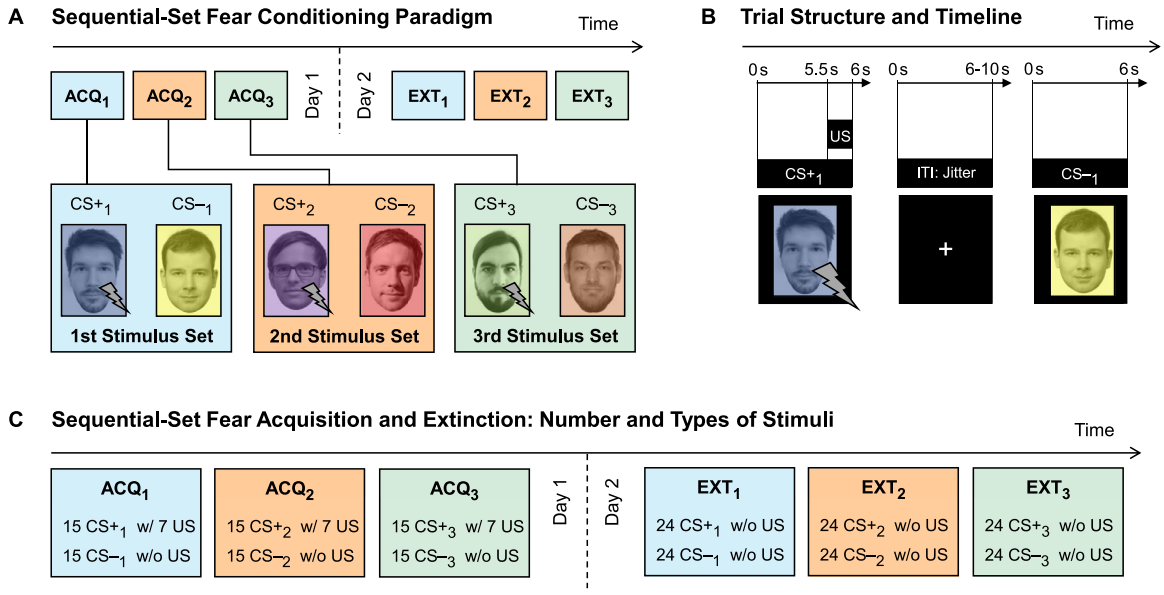

Fig. 1. Sequential-set fear conditioning paradigm. (A) Participants underwent three successive acquisition training phases (ACQ_1, ACQ_2, and ACQ_3), each with a novel conditioned stimulus (CS+/CS-) set (differently tinted neutral faces). For instance, the $CS+_1/CS-_1$ stimulus set was used during the first acquisition training (ACQ_1). Approximately 24 h after fear conditioning, subjects underwent sequential-set extinction, which consisted of three successive extinction training phases (EXT_1, EXT_2, and EXT_3), each with the corresponding CS+/CS- set. (B) Trial structure and timeline for a single CS trial. All CSs were presented for 6 s, followed by a jittered 6–10 s intertrial interval. During acquisition training, CS+ were paired with an aversive electric shock unconditioned stimulus (US). The delivery of the US started 500 ms before the CS offset. (C) The number and stimuli types presented during the experimental phases. During three acquisition training phases, $CS+_1$, $CS+_2$, and $CS+_3$ were reinforced with an aversive US ("w/", reinforcement rate of 47%), while $CS-_1$, $CS-_2$, and $CS-_3$ were never paired with a US ("w/o"). Note: Due to licensing restrictions, we cannot publish the original stimulus material that we used in the present study. To illustrate the paradigm, panels A and B contain comparable stimuli with faces of the authors of this article and their colleagues. The original stimulus material is available upon request.

Under the assumption of a significance level of $\alpha = .05$ and a power level of $1 - \beta = .08$, *a priori* power analyses revealed that a total sample size of 19 would be required. To allow for a potential data loss of up to 20%, we recruited 24 participants.

All subjects gave written informed consent to participate. They participated for either partial fulfillment of course credit or were reimbursed with €10 per hour. Exclusion criteria were a history of cardiovascular, neurological, or mental disorders (assessed by the short version of the Diagnostic Interview for Mental Disorders, Mini-DIPS; Margraf, 1994), and regular use of either illegal drugs or prescription drugs that affect the central nervous system. All participants were asked to refrain from alcoholic or caffeinated drinks, heavy meals, and strenuous exercise prior to the experiment. The study protocol was approved by the local ethics committee of the Department of Medicine at the University of Marburg.

2.2. Experimental fear conditioning and extinction paradigm

We developed a new experimental paradigm that is designed to study the time course of electrocortical fear responses throughout fear conditioning and extinction. Our paradigm was administered over two consecutive days (see Fig. 1): Participants underwent sequential-set fear conditioning on day 1, while sequential-set fear extinction took place 24 h later on day 2. Specifically, one CS (the CS+) was paired with the aversive US, while a second CS (the CS-) remained unpaired. The differential fear response can be quantified as heightened responses to CS+ compared with CS-.

Importantly, day 1 sequential-set fear conditioning consisted of three successive acquisition training phases, each with a novel CS+/CS- set. Here, we denote each of the three sets with a subscript (i.e., $CS+_1/CS-_1$, $CS+_2/CS-_2$, and $CS+_3/CS-_3$). Specifically, each set comprised two different neutral faces on different background colors, which served as CS+ and CS-, respectively. During the acquisition training of set 1 (ACQ_1), the $CS+_1/CS-_1$ stimulus set was used, during the acquisition training of set 2 (ACQ_2), a second $CS+_2/CS-_2$ stimulus set was shown, and during the last acquisition training (ACQ_3), a third $CS+_3/CS-_3$ stimulus set was presented. During each of the three acquisition training phases, CS+ and CS- stimuli were shown 15 times each in a random order, with the CS+ paired with an aversive electric shock US during 7 trials (see Fig. 1C). To familiarize participants with the stimuli, the corresponding CS+ and CS- stimuli were presented four times during three pre-acquisition phases (pre-ACQ), which took place immediately before the respective acquisition training phase. Prior to acquisition, participants were verbally instructed to expect a shock paired with the presentation of the CS+ face (Hollandt et al., 2020). Participants were instructed about the contingency but were not informed about the reinforcement rate (Mertens et al., 2018). Note that the first CS+ during each acquisition phase was always paired with the US.

Approximately 24 h after fear conditioning, subjects underwent sequential-set fear extinction. Similar to the acquisition training procedure, day 2 fear extinction consisted of three successive extinction training phases, each with the corresponding CS+/CS- set. Specifically, during each extinction training (EXT_1, EXT_2, and EXT_3), CS+ and CS- stimuli were presented 24 times each in a random order without any US presentation (see Fig. 1C). To reactivate the CS-US contingency, a single CS+ reinforced with the electric shock US (same intensity as during day 1) and a single CS- were presented prior to each extinction training phase (Monfils and Holmes, 2018; Hollandt et al., 2020). On day 2, participants were instructed that electric shock stimuli "may occur" during the experiment.

Consistent with several studies from human (e.g., Feng et al., 2015; Ebrahimi et al., 2020; Hollandt et al., 2020) and animal (e.g., Voulo and Parsons, 2017; Ramanathan et al., 2018; Hartley et al., 2019) literature, fear conditioning and extinction were separated by approximately 24 h to allow fear memory consolidation prior to extinction training. There is robust evidence that sleep plays a pivotal role in the consolidation of fear-conditioned memories (Kumar et al., 2012; Pace-Schott et al., 2015; Menz et al., 2016). If fear conditioning and extinction are performed on

the same day, extinction learning is likely to interfere with fear memory consolidation, resulting in the so-called "immediate extinction deficit" (Maren, 2014).

2.3. Conditioned and unconditioned stimuli

Following previous studies, pictures of male faces with a neutral expression and tinted in either blue, yellow, purple, red, green, or orange color (as used by Klumpers et al., 2010; Duits et al., 2017; Heinig et al., 2017; Hollandt et al., 2020; see Fig. 1A and B) served as CSs. Photos of six male faces were selected from the Psychological Image Collection at Stirling (http://pics.psych.stir.ac.uk; nottingham_scans set; ids: m025, m051, m064, m095) and from the NimStim stimulus set (Tottenham et al., 2009; ids: 27M_NE_C, 36M_NE_C). All CS faces (size: 13.5 × 18 cm) were presented on a 24-in computer monitor placed approximately 50 cm in front of the participant. The stimuli were shown for 6 s, using the computer program Presentation 18.2 (Neurobehavioral Systems, Berkeley, CA/USA). During a jittered intertrial interval (defined as CS offset to CS onset) of 6–10 s, a white fixation cross was shown on a black background (see Fig. 1B). The assignment of face stimuli to CS+ and CS- was counterbalanced.

The US consisted of a 500-ms multipulse (100 single 5-ms pulses) percutaneous electrical stimulation. US presentation started 5.5 s after CS onset. Electrical stimulation was delivered from a constant current stimulator (DS7A; Digitimer Ltd., Welwyn Garden City, UK; 400 V maximal voltage) using two steel disk electrodes (Technomed Europe, Maastricht, The Netherlands; 8-mm diameter, 23 mm apart) attached to the inside of the left forearm, about 11 cm from the carpus. During a work-up procedure (a detailed protocol is available at https://doi.org/10.5281/zenodo.4294603), we presented electrical stimuli at increasing intensities until the shocks were subjectively perceived as "difficult to bear, but acceptable" ($M = 1.84$ mA, $SD = 0.76$ mA). Using these relatively high shock intensities, we have already shown successful fear conditioning on physiological and subjective levels in a previous simultaneous EEG-fMRI study (Sperl et al., 2019). Shock electrodes were attached during all experimental stages.

2.4. Subjective CS ratings

Prior to and after each experimental stage, participants were asked to indicate the US expectancy ("How likely is it that the electric stimulus will coterminate with this picture?") for each CS on an 11-point numeric rating scale ranging from 0% ("US will definitely not coterminate") to 100% ("US will definitely coterminate"). Furthermore, participants rated the subjective valence ("How good or bad do you feel when looking at this picture?"; 0 = "very good" to 100 = "very bad") and arousal ("How aroused do you feel when looking at this picture?"; 0 = "not aroused at all" to 100 = "extremely aroused") of their current feeling on an 11-point scale. In order to assess the temporal dynamics of extinction learning over trials (Golkar et al., 2013), each extinction training phase was split into three blocks. Additional subjective CS ratings were obtained between these blocks.

2.5. EDA data acquisition and analyses

Electrodermal activity (EDA), electrocardiogram (ECG), and electroencephalogram (EEG) were recorded at a 1024 Hz sampling rate using the BioSemi Active Two EEG system (BioSemi, Amsterdam, The Netherlands). Physiological data were low-pass filtered online with a cutoff frequency of 208 Hz. For EDA (exosomatic measurement, 1 μA at 16 Hz AC), two Ag/AgCl electrodes (5-mm diameter) filled with isotonic (0.5% NaCl) electrolyte medium were placed on the thenar and hypothenar eminences of the nondominant (left) hand. Raw EDA data were low-pass filtered (1 Hz, signal amplitude is attenuated by 3 dB at cutoff frequency, 4th order Butterworth filter, 24 dB/octave roll-off) offline in BrainVision Analyzer 2.1.2 (Brain Products, Munich, Germany) and downsampled to 128 Hz. Artifact correction and trough-to-peak analyses were performed in Ledalab 3.4.9 (Benedek and Kaernbach, 2010a, 2010b), implemented in MATLAB 9.2 (MathWorks, Natick, MA/USA). After visual data inspection, technical artifacts were corrected with spline or cubic interpolation. For each CS trial, a skin conductance response (SCR) score was calculated as the amplitude-sum of significant SCRs within 1 and 5 s after the CS onset. Given that latencies shorter than 1 s should be treated with caution (Boucsein et al., 2012), SCRs during the first second after CS onset were omitted. SCRs smaller than 0.01 μS were considered zero responses. To obtain a normal distribution, SCR scores were logarithmized, $\ln(\mu S+1)$, before averaging. SCR scores were then averaged across trials for each CS type. Both unreinforced and reinforced CS+ trials were included because the SCR response window did not overlap with the US onset. In fact, the trial timing of our paradigm was optimized to also allow for appropriate SCR and heart period (see below) analyses. The CSs were shown for 6 s, and the US coterminated with the last 500 ms of the CS+. Compared with the majority of fear conditioning studies, the CS duration is relatively long. However, this timing ensures that the SCR response window does not overlap with the US onset. SCR amplitudes typically habituate over time, and habituation is usually weaker for CS+ versus CS- (Lonsdorf et al., 2017). If only unreinforced SCR trials are included in statistical analyses, different habituation curves for CS+ compared with CS- can be problematic and reduce statistical power. In the present study, we circumvented this shortcoming, and *all* trials could be used for statistical analyses. In addition to CS-evoked SCRs, we also analyzed responses to the US.

2.6. ECG data acquisition and analyses

For ECG, two Ag/AgCl electrodes (4-mm diameter) were filled with liquid gel and placed in Lead II configuration (right arm and left leg). Preprocessing of ECG data was performed in BrainVision Analyzer 2.1.2 (Brain Products, Munich, Germany). Raw ECG data were band-pass filtered (1–30 Hz, signal amplitude is attenuated by 3 dB at cutoff frequencies, 4th order Butterworth filter, 24 dB/octave roll-off) and notch filtered (50 ± 2.5 Hz, 16th order Butterworth filter, 96 dB/octave roll-off) offline. Afterward, R-spikes were detected automatically with the ECG Markers Solution in BrainVision Analyzer 2.1.2 (Brain Products, Munich, Germany), ECG data were manually checked for artifacts, and R-spikes were corrected if necessary. Then, we calculated a continuous heart period trace using custom-made MATLAB scripts (MATLAB 9.2; MathWorks, Natick, MA/USA). In particular, the ECG was converted to a time course of interbeat intervals (IBIs), and each IBI time point represents the latency between the pre- and succeeding R-spike in ms (Mueller et al., 2013). Next, this IBI time series was segmented into epochs ranging from −1 to 10 s relative to the onset of the CS, baseline-corrected relative to 1 s pre-CS, and averaged across trials for each CS type. In fear conditioning experiments, heart rate responses to a CS typically display a three-phasic response pattern (Lipp, 2006), starting with an initial heart rate deceleration (D1), followed by a transient acceleration (A) and a second deceleration (D2). Fear conditioning evokes a larger second deceleration component for CS+ compared with CS- (Notterman et al., 1952; Deane and Zeaman, 1958; Panitz et al., 2015). To analyze CS-evoked fear bradycardia, the maximum IBI value for the D2 period, which typically coterminates with the onset of the US (Deane and Zeaman, 1958), was extracted from 4 to 8 s after CS onset. If the distance between CS and US onsets is too short, fear-conditioned deceleration effects can be attenuated by the preceding acceleration component. As described above, we used a rather long CS-US interval, which allows to reliably disentangle decelerative (D2) from accelerative (A) cardiac responses. Fear bradycardia usually overlaps with the US onset (Deane and Zeaman, 1958). To avoid contamination by an evoked response to the US, we included only unreinforced CS+ trials for the

acquisition training phases. This approach allowed us to extend the response window beyond the US onset. We also analyzed unconditioned responses, which typically appear as cardiac acceleration (A) to an electrotactile US (Ginsberg and Thysell, 1966; Lipp, 2006; Vila et al., 2007). To quantify acceleratory responses, the minimum IBI value within 4 s after US onset was extracted.

2.7. EEG data acquisition and analyses

EEG was recorded using a 64-channel BioSemi Active Two EEG system (BioSemi, Amsterdam, The Netherlands), referenced to the common mode sense (CMS) electrode in a dynamic feedback loop with the driven right leg (DRL) electrode. The electrodes contained a sintered Ag/AgCl electrode tip. A schematic illustration of the electrode montage and scalp coordinates are available at https://doi.org/10.5281/zenodo.4294603. EEG data were preprocessed in BrainVision Analyzer 2.1.2 (Brain Products, Munich, Germany). Raw EEG data were high-pass filtered (0.5 Hz, signal amplitude is attenuated by 3 dB at cutoff frequency, 4th order Butterworth filter, 24 dB/octave roll-off) and notch filtered (50 ± 2.5 Hz, 16th order Butterworth filter, 96 dB/octave roll-off) offline. The high-pass filter was applied to remove slow drifts, which can be caused by skin potentials (Cohen, 2014), and is required for independent component analysis (ICA) to obtain reliable and valid decomposition results (Winkler et al., 2015; Dimigen, 2020). We confirmed that all results could be reproduced with different high-pass filter settings (0.5 Hz, 0.1 Hz, 0.01 Hz, no high-pass filter; see Supplementary Material A). ICA (extended infomax ICA with classic principal component analysis sphering on the whole artifact-free EEG dataset) was used for eye-blink/movement correction, and corrupted channels were interpolated using a spherical spline interpolation (Perrin et al., 1989). Afterward, data were re-referenced to the average reference. In accordance with prior research (Auerbach et al., 2016; Whitton et al., 2016; Seligowski et al., 2018; Schroder et al., 2019), we used a semi-automated procedure to reject artifact intervals, using the following criteria: (a) a voltage step exceeding 50 μV between two contiguous sampling points, (b) an absolute voltage difference of more than 150 μV within a period of 200 ms, (c) an absolute amplitude lower than −75 μV or higher than 75 μV, and (d) a maximum voltage difference of less than 0.5 μV between the maximum and minimum within a period of 100 ms. In addition to these semi-automated artifact rejection procedures, the EEG signal was visually inspected for manual artifact identification and removal by an experienced rater. All intervals that contained artifacts in at least one channel were discarded from further EEG analyses. Information on the residual number of trials per CS type after artifact rejection is provided in Supplementary Material B (see Supplementary Fig. S2). Finally, EEG was low-pass filtered (30 Hz, signal amplitude is attenuated by 3 dB at cutoff frequency, 4th order Butterworth filter, 24 dB/octave roll-off), and we computed ERPs covering 1000 ms time-locked to the CS+ and CS- onsets. ERPs were baseline-corrected (200 ms pre-stimulus) and averaged across trials. We included unreinforced and reinforced CS+ trials, as EEG epochs ended before the US onset. In addition to responses to the CSs, we also assessed US-evoked ERPs.

Traditionally, ERP analyses have mostly applied standard univariate statistics and compared waveforms at certain channels during certain time windows, often based on previous literature. However, this approach neglects the intrinsic correlation structure of EEG data (Koenig and Melie-García, 2009; Michel and Murray, 2012), which is related to redundancy in space (correlation of neighboring electrodes) and time (correlation of neighboring sampling points). This can be problematic, as traditional methods thereby often miss out on a large amount of the information which can be obtained from the EEG signal. To tackle this problem, we applied the so-called topographic analysis of variance (TANOVA) method (Murray et al., 2008; Koenig and Melie-García, 2009) in the present study. While retaining statistical rigor, this multivariate approach considers spatial and temporal information from each sensor and sampling point, respectively (Michel and Murray, 2012).

Furthermore, the exact time windows for statistical analyses on ERP differences between experimental conditions has often been selected "based on prior research" (Keil et al., 2014). However, in the fear conditioning literature, investigated ERP components (e.g., C1, P1, LPP) and exact latency windows vary strongly among studies (Pizzagalli et al., 2003; Miskovic and Keil, 2012; Ferreira de Sá et al., 2019). In addition, Luck and Gaspelin (2017) argued that the latency of observed effects may differ between studies due to low-level sensory factors (e.g., stimulus luminance and discriminability) which are often not standardized between fear conditioning studies. These discrepancies turn the selection of ERP components and adequate measurement windows into a somewhat speculative endeavor which can lead to biased conclusions (Michel and Murray, 2012; Keil et al., 2014; Clayson et al., 2019). Given these circumstances, the appropriate selection of relevant time windows is particularly challenging when validating a new paradigm. Moreover, if time windows for ERP analyses are selected *a priori*, effects (e.g., during periods of low-amplitude) can easily be overlooked (Murray et al., 2008).

Therefore, we applied a *data-driven* approach that aims to assess differences in amplitude strength and topography between experimental conditions. In contrast to "traditional" ERP analyses, this method provides a more complete insight and does not suffer from an *a priori* bias with regard to time windows and electrode locations (Murray et al., 2008; Michel and Murray, 2012). To identify relevant ERP components that are modulated by the processing of CS+ compared with CS-, we analyzed scalp ERP data with spatio-temporal electric field analyses (Lehmann and Skrandies, 1984; Murray et al., 2008). Importantly, because we were specifically interested in different electrocortical processing between CS+ and CS-, our goal was to identify continuous periods during which ERPs and topographic maps significantly differ between both CS types (Gianotti et al., 2008; Murray et al., 2008; Koenig and Melie-García, 2009). The data-driven TANOVA method tests for map differences between conditions and provides a *p*-value for each time point of the ERP trace, quantifying the strength of the difference map between CS+ and CS- conditions (Murray et al., 2008; Koenig and Melie-García, 2009). Consequently, time windows of contiguous time points with significant TANOVA results ($p \leq .05$) indicate ERP components that are modulated by CS+ compared with CS-. This method has previously been used to identify relevant ERP components that reflect differential electrocortical processing between experimental conditions (e.g., Lavric et al., 2004; Maurer et al., 2005; Martinovic et al., 2014; Bailey et al., 2019).

We performed a TANOVA as implemented in the Randomization Graphical User Interface (RAGU) software package (version 2018-10-16; Koenig and Melie-García, 2009; Koenig et al., 2011; Habermann et al., 2018), which is based on MATLAB 9.2 (MathWorks, Natick, MA/USA), to compare scalp field differences between CS+ and CS- across all EEG channels and time points. Specifically, RAGU performs randomization statistics without making any *a priori* assumptions concerning electrode sites or time windows. To obtain an accurate estimate of significance at the 5% level, 5000 randomization runs were performed (Manly, 2007; Koenig et al., 2011; Habermann et al., 2018). We used global duration statistics to control for multiple comparisons among time points. Therefore, the probability for a given effect duration under the null hypothesis is calculated. This analysis indicates a *minimum effect duration* containing time points with $p \leq .05$ that needs to be exceeded to reach "overall" significant TANOVA effects. Note that this approach efficiently controls for multiple comparisons among time points but results in highly conservative significance testing (Habermann et al., 2018). In particular, periods of early ERP effects are often short-lasting and therefore less likely to meet the critical duration for reaching significance at this "overall" level. Global duration statistics revealed a duration of 56.64 ms for acquisition and 63.48 ms for extinction, respectively. For day 1 fear conditioning, ERPs were averaged across all acquisition training trials and

all three stimulus sets to compute the TANOVA. For day 2 fear extinction, we expected a large conditioned response during the first trials and a decline toward later trials. Hence, ERPs from only the first extinction training block (i.e., the first 8 trials) from all three stimulus sets were averaged for the extinction TANOVA. In addition, we computed follow-up ANOVAs to explicitly test for the stability of the observed ERP effects across CS sets. The mean voltage following CS+ and CS- presentations within the time windows that were indicated by TANOVA was extracted separately for each stimulus set and subjected to *Contingency* x *Set* x *Channel* (x *Hemisphere*) ANOVAs. For follow-up statistical analyses, channels with the largest negative or positive deflection in the grand-grand average ERP (across CS+ and CS- trials and stimulus sets) were used.

Map differences between conditions can be produced (1) by a change in strength of similar generators ("quantitative difference of activation"), (2) by differences in source orientation or distribution ("qualitative difference"), or (3) by a combination of both (Koenig and Melie-García, 2009; Koenig et al., 2011; Habermann et al., 2018). In the present study, we were interested in both quantitative and qualitative differences between maps. Thus, our TANOVA approach tested for both effects, which offers the possibility to detect *all* (i.e., strength- *and* topography-related) systematic electrocortical differences between CS+ and CS- (Maurer et al., 2005). If, however, EEG data are normalized prior to spatio-temporal TANOVA analyses, significant map effects indicate that partially different sources ("qualitative difference") gave rise to scalp differences between conditions (Michel and Murray, 2012). To explicitly test for the influence of different generators in the brain, we also computed a second TANOVA on the amplitude-normalized maps (Koenig and Melie-García, 2009; Koenig et al., 2011; Habermann et al., 2018). To achieve data normalization, all potential values of a specific map were divided by its Global Field Power (GFP), i.e., all maps were scaled to have GFP = 1. The GFP, which is calculated as the mean absolute potential difference in the field, represents the spatial standard deviation across all electrodes at a specific time point and is considered to be a reference-free measure of response strength (Lehmann and Skrandies, 1980). Detailed analyses on amplitude-normalized maps are shown in Supplementary Material C.

2.8. Statistical analyses

For all dependent variables, we computed repeated-measures ANOVAs with the factors "*Contingency*" (CS+ versus CS-) and "*Set*" (CS set 1, 2, 3; e.g., $CS+_1/CS-_1$). Day 1 conditioning and day 2 extinction were analyzed separately.

For fear conditioning, subjective ratings (valence, arousal, and US expectancy) collected after each acquisition training phase (ACQ_1, ACQ_2, and ACQ_3) were used for ANOVAs. Similarly, we used the condition-specific average for the skin conductance response, fear bradycardia, and EEG amplitude at previously identified spatio-temporal positions (see above). The validity of the sequential-set fear conditioning paradigm would be supported by greater fear responses (i.e., higher subjective ratings, larger physiological responses) for CS+ compared with CS-, which should be comparable across all three CS sets. Thus, an increase in conditioned responses throughout fear acquisition training phases can be interpreted as successful fear conditioning.

For fear extinction, we expected a decline in conditioned responses. Hence, the factor "*Time*" referred to the affective CS ratings *before* and *after* each extinction training block, or to the mean physiological CS-evoked response *during* the respective extinction training block. For ratings and EEG data, these blocks consisted of eight trials. Due to a better signal-to-noise ratio for EDA and ECG versus EEG data (e.g., Panitz et al., 2015; Sperl et al., 2016), only four trials were averaged for peripheral physiological data to allow for more fine-grain analyses of extinction learning over time. The validity of sequential-set fear extinction would be supported by a decline in conditioned fear responses from early to late blocks for CS sets 1, 2, and 3.

Our overarching goal was to develop a paradigm that allows studying learning dynamics of neural responses within experimental stages. To account for more subtle changes in ERP responses across trials, experimental stages were split into smaller sub-blocks of five (acquisition training) or four (pre-acquisition and extinction training) trials each. Collapsing across CS sets allowed us to probe temporal changes in neural responding across trials. Importantly, this approach leads to (a) an adequate signal-to-noise ratio (through averaging *across* CS sets) while (b) creating the possibility to detect temporal changes during learning (because only a few trials need to be averaged *within* each CS set). Mean ERP responses (averaged across CS sets and across EEG channels with significant effects) were subjected to ANOVAs, including the factors "*Contingency*" (to compare CS+ with CS-) and "*Sub-Block*" (to assess temporal changes during learning). We expected an increase in conditioned electrocortical responses from early to late conditioning. Conversely, differential responses should decline throughout fear extinction. Polynomial contrasts were calculated for the *Contingency* x *Sub-Block* interactions to evaluate whether the increase (during fear conditioning) and decrease (during fear extinction) can be best described by a linear, quadratic, or cubic trend.

Significant ANOVA interactions involving the factor *Contingency* (CS+ versus CS-) were further analyzed using follow-up ANOVAs and *t*-tests. Statistical tests on physiological data (EDA, ECG, and EEG) and subjective data (subjective CS ratings of arousal, valence, and US expectancy) were performed using SPSS 24 for Windows (IBM, Armonk, NY/USA). To reach statistical significance, $p \leq .05$ was required. The Greenhouse-Geisser (1959) correction was applied for repeated-measures ANOVAs when the sphericity assumption was not met. Cohen's (1988, 1992) *d* is used to report the effect size of conditioned fear responses.

2.9. Data and code availability

De-identified data for analyses described in this manuscript along with a code-book and the data analysis scripts are publicly posted at https://doi.org/10.5281/zenodo.4294603, and are available online for interested readers.

3. Results

3.1. Subjective ratings and peripheral physiological data during fear acquisition

As expected, ANOVAs on subjective ratings of the CS after acquisition training confirmed successful fear conditioning (*Contingency* main effect, see Fig. 2A) with regard to valence, arousal, and US expectancy both across ($F(1,20) \geq 123.82$, all $ps \leq .001$, $ds \geq 2.43$) and within different CS+/CS- sets ($F(1,20) \geq 8.45$, all $ps \leq .001$). Supporting successful fear learning at the electrodermal level (see Fig. 2B), participants showed significant SCR increases to the CS+ compared with the CS- (*Contingency* main effect, $F(1,20) = 30.53$, $p < .001$, $d = 1.21$). Moreover, individual paired-samples *t*-tests (CS+ versus CS-) for CS set 1 ($t(20) = 6.48$, $p < .001$), set 2 ($t(20) = 5.37$, $p < .001$), and set 3 ($t(20) = 4.39$, $p < .001$) demonstrated higher SCRs during all three successive acquisition training phases. In line with affective CS ratings and elevated SCRs, a significant *Contingency* main effect ($F(1,20) = 21.72$, $p < .001$, $d = 1.17$) for heart period data indicated a successful acquisition of fear-conditioned bradycardia. Specifically, Fig. 2C shows that CS+ evoked a stronger cardiac deceleration compared with CS-. Moreover, *t*-tests within CS sets confirmed fear-conditioned bradycardia for CS set 1 ($t(20) = 3.76$, $p = .001$), set 2 ($t(20) = 4.16$, $p < .001$), and set 3 ($t(20) = 3.78$, $p = .001$).

In addition to conditioned fear responses, we were also interested in unconditioned physiological responses (see Fig. 2B and C). The US evoked significant SCRs (one-sample *t*-test, $\mu \neq 0$) during the acquisition training of CS set 1 ($t(20) = 6.60$, $p < .001$), set 2 ($t(20) = 5.99$, $p < .001$), and set 3 ($t(20) = 5.76$, $p < .001$). Furthermore, we observed

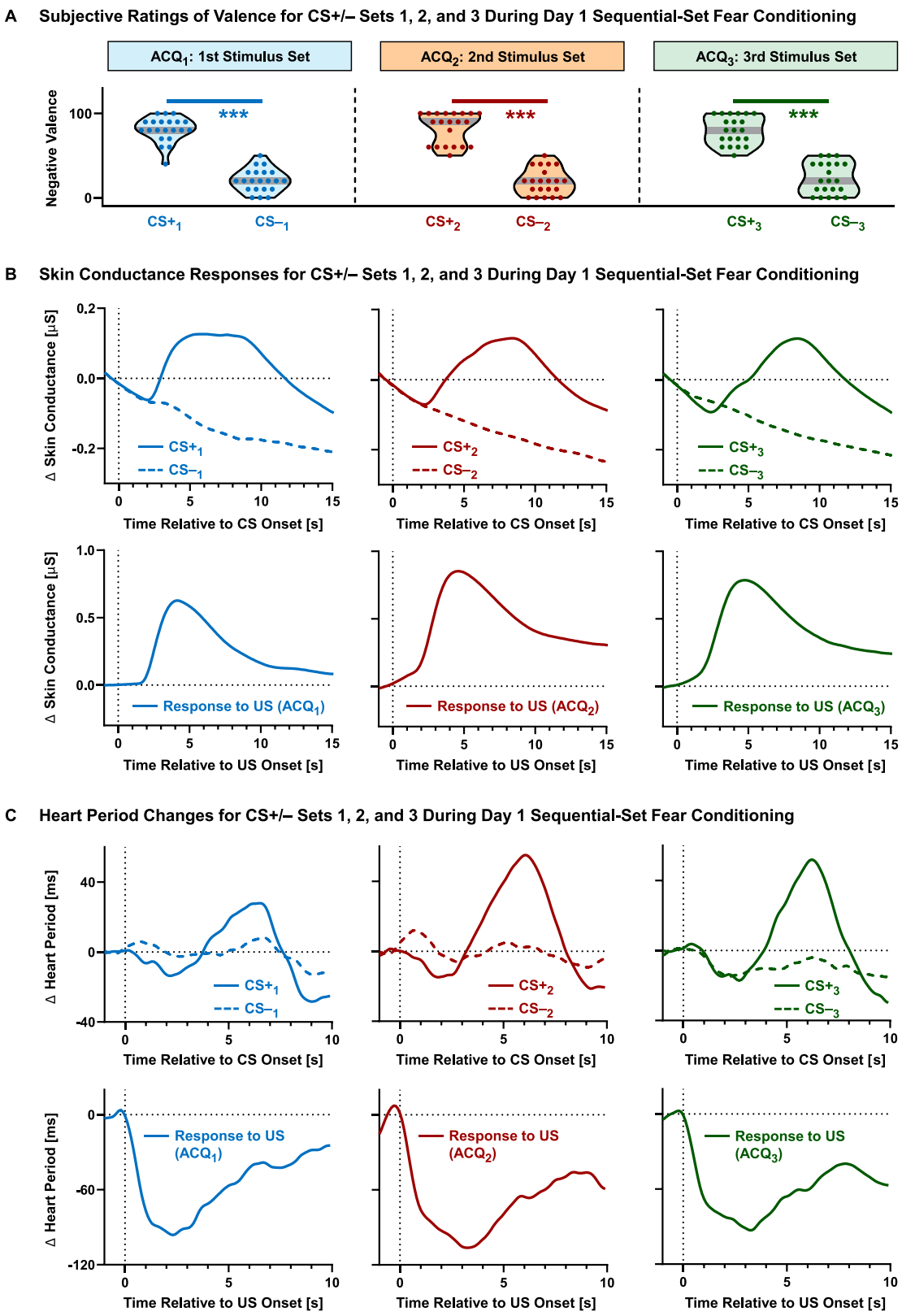

Fig. 2. Subjective and peripheral physiological (electrodermal activity and electrocardiogram) correlates of day 1 sequential-set fear conditioning. Conditioned and unconditioned responses are plotted separately for the three conditioned stimulus (CS) sets (e.g., $CS+_1$ versus $CS-_1$). (A) Ratings of CS-associated valence indicated that CS+ compared with CS- was associated with more negative valence for all three CS sets. Participants were asked to indicate their current feeling (0 = "very good" to 100 = "very bad") when looking at the faces. The results for ratings of arousal and US expectancy were similar. Violin plots display the frequency distribution of subjective ratings. Individual data points are superimposed on the violin plot, and the median is displayed as a grey horizontal line. (B) Furthermore, the CS+ (versus CS-, upper panels) and the US (lower panels) evoked increased skin conductance response (SCR) amplitudes. (C) While the CS+ (versus CS-, upper panels) is associated with relative fear bradycardia (i.e., heart period slowing), cardiac responses to the US (lower panels) showed a large acceleration component. Peripheral physiological responses were similar for all three stimulus sets. To illustrate the time series of CS- and US-evoked changes in peripheral physiological data, SCR and heart period data (interbeat intervals) were baseline-corrected (1 s pre-CS) and averaged across trials and participants. Only unreinforced CS+ trials were averaged, which allowed us to display physiological responses beyond the US onset. $^{***}p \leq .001$.

cardiac acceleration to the US during the first ($t(20) = -8.68$, $p < .001$), second ($t(20) = -7.42$, $p < .001$), and third ($t(20) = -8.08$, $p < .001$) acquisition training. Electrodermal (see Fig. 2B) and cardiac (see Fig. 2C) unconditioned responses did not habituate and were similar for each of the three CS sets (no repeated-measures effects of *Set*, all $ps \geq .554$).

3.2. Subjective ratings and peripheral physiological data during fear extinction

The *Contingency* x *Set* x *Time* ANOVAs for subjective CS ratings during the extinction stage revealed significant *Contingency* x *Time* interactions for valence ($F(3,60) = 48.45$, $p < .001$), arousal ($F(3,60) = 49.52$, $p < .001$), and US expectancy ($F(3,60) = 59.58$, $p < .001$) ratings, indicating successful extinction learning for each CS set (see Fig. 3A). Although differences between CS+ and CS- were not completely absent at the end of extinction training, differential fear responses diminished in each of the three CS sets. Analyses of CS-evoked SCRs ($F(10,200) = 4.26$, $p = .002$) and heart period changes ($F(10,200) = 2.56$, $p = .006$) yielded significant *Contingency* x *Time* x *Set* interactions, reflecting extinction learning (decline of conditioned responses across trials) and habituation (decline across CS sets). As intended, the transition to trials of a new CS set induced "dishabituation", and we observed a successful fear recall during early extinction training for each CS set. Specifically, SCRs (see Fig. 3B) were elevated for CS+ compared with CS- during the first four extinction training trials for CS set 1 ($t(20) = 5.68$, $p < .001$), set 2 ($t(20) = 3.90$, $p = .001$), and set 3 ($t(20) = 2.27$, $p = .035$). Likewise, CS+ versus CS- was associated with cardiac deceleration (see Fig. 3C) during the first four extinction training trials for CS set 1 ($t(20) = 2.22$, $p = .038$), set 2 ($t(20) = 3.62$, $p = .002$), and set 3 ($t(20) = 2.28$, $p = .034$).

3.3. EEG: ERP components during fear acquisition

For day 1 fear conditioning, the TANOVA identified three time windows with continuously significant differences between ERP maps for CS+ and CS- (see Fig. 4A): (a) 33–60 ms[1] (b) 108–200 ms, and (c) 468–820 ms after CS onset. Supporting its validity, our data-driven approach tapped into latencies that overlap with periods reported in previous fear conditioning studies with "traditional" EEG methods (Miskovic and Keil, 2012; Panitz et al., 2015, 2018). A second TANOVA on the amplitude-normalized maps (see Supplementary Material C) indicated that different intracranial brain generators contributed to the effects in each of the three time windows (see Supplementary Fig. S3).

33–60 ms post-CS. As indicated in Fig. 4A, the TANOVA for conditioning revealed significant differences between ERP maps following CS+ versus CS- as early as 33 to 60 ms after stimulus onset (averaged across the significant time window: TANOVA $p = .006$). The grand-grand average ERP (across CS+ and CS- trials and stimulus sets) showed a widespread negativity at centro-parietal electrode sites, in particular at channels CP1, CPz, CP2, P1, Pz, P2, and POz. Thus, mean voltages at these channels were used to compute a *Contingency* x *Set* x *Channel* follow-up ANOVA. A significant *Contingency* main effect ($F(1,20) = 4.70$, $p = .042$) confirmed more negative ERP amplitudes for CS+ compared with CS- (see Fig. 4B). Separate *Contingency* x *Channel* ANOVAs for each of the three CS sets did not reach significance (all $ps \geq .102$). This outcome mirrors previous findings that fear conditioning effects on short-latency sensory processing require a massive number of acquisition trials to be detected (Stolarova et al., 2006; Miskovic and Keil, 2012; Thigpen et al., 2017).

108–200 ms post-CS. The TANOVA further revealed significant differences between CS+ and CS- maps from 108 to 200 ms after stimulus onset (averaged across the significant time window: TANOVA $p < .001$, see Fig. 4A), which survived the duration threshold. This time window comprises a relatively long period, containing a short positive deflection and followed by a more sustained negative deflection. This response pattern was particularly pronounced over occipito-temporal electrode sites. A follow-up ANOVA was conducted at occipito-temporal channels over the left (T7, C5, TP7, CP5, P7, P5, PO7, PO3) and right (T8, C6, TP8, CP6, P8, P6, PO8, PO4) hemisphere. Including the factor *Hemisphere* allowed us to control for lateralization (Caharel et al., 2009; Rossion and Jacques, 2012). This *Contingency* x *Set* x *Channel* x *Hemisphere* ANOVA yielded a significant *Contingency* main effect ($F(1,20) = 23.91$, $p < .001$) and a significant *Contingency* x *Channel* interaction ($F(7,140) = 10.17$, $p < .001$). The effects were comparable across CS sets and hemispheres (ps for interactions $\geq .266$). To further assess the significant interaction with the factor *Channel*, paired-samples *t*-tests were computed for individual EEG channels and indicated a more negative ERP amplitude for CS+ compared with CS- at CP5 ($p = .008$), P7 ($p < .001$), P5 ($p < .001$), PO7 ($p = .001$), and PO3 ($p = .001$) over the left hemisphere, as well as at P8 ($p = .014$), P6 ($p < .001$), PO8 ($p < .001$), and PO4 ($p < .001$) over the right hemisphere (see Fig. 4C). In addition, we computed follow-up *Contingency* x *Channel* x *Hemisphere* ANOVAs for individual CS sets (see Fig. 5A). For the first CS set, a significant *Contingency* main effect ($F(1,20) = 6.06$, $p = .023$) and a significant *Contingency* x *Channel* interaction ($F(7,140) = 3.76$, $p = .030$) confirmed more negative amplitudes for CS+ versus CS-, particularly at parietal and parieto-occipital channels. Likewise, the ANOVA for the second CS set yielded a significant *Contingency* main effect ($F(1,20) = 10.66$, $p = .004$) and a significant *Contingency* x *Channel* interaction ($F(7,140) = 4.99$ $p = .016$). Finally, there was a significant *Contingency* main effect for the third CS set ($F(1,20) = 4.72$, $p = .042$). In summary, our data emphasize the acquisition of a robust conditioned electrocortical response during this period for each of the three CS sets.

468–820 ms post-CS. Finally, the TANOVA showed that CS+ and CS- maps significantly differed from 468 to 820 ms after stimulus onset (averaged across the significant time window: TANOVA $p < .001$, see Fig. 4A), which also survived the duration threshold for overall significance. During this period, the grand-grand average ERP showed strong and sustained positivity at parieto-occipital electrode sites. For follow-up statistical analyses, parieto-occipital channels P1, Pz, P2, PO3, POz, PO4, O1, Oz, and O2 were used, where the late positive voltage deflection was maximal. The follow-up ANOVA including the factors *Contingency* x *Set* x *Channel* revealed a significant *Contingency* main effect ($F(1,20) = 15.24$, $p = .001$), indicating a larger positive deflection for CS+ compared with CS-, as shown in Fig. 4D. Paired-samples *t*-tests confirmed significant effects for all individual EEG channels that were included in the ANOVA. ERP effects in this time window were comparable across CS sets (ps for interactions $\geq .129$). Furthermore, to explicitly confirm ERP modulations for all stimulus sets, separate follow-up *Contingency* x *Channel* ANOVAs for individual CS sets were carried out (see Fig. 5B). We confirmed a significantly larger positive deflection for CS+ compared with CS- for CS set 1 (*Contingency* x *Channel* interaction, $F(8,160) = 2.58$, $p = .044$), set 2 (*Contingency* main effect, $F(1,20) = 16.75$, $p = .001$), and set 3 (*Contingency* main effect, $F(1,20) = 6.22$, $p = .021$).

ERPs evoked by the US. During acquisition training, the US was associated with a robust negative deflection from 50 to 200 ms (see Fig. 6A), followed by a positive deflection from 200 to 350 ms after stimulus onset (see Fig. 6B). The initial negativity was largest at fronto-central channels FC1, FCz, FC2, C1, Cz, and C2 (see Fig. 6C). A *Channel* x *Set* ANOVA

[1] Although this period did not exceed the more conservative duration criterion, previous literature (e.g., Stolarova et al., 2006; Hintze et al., 2014; Mueller and Pizzagalli, 2016; Thigpen et al., 2017) leads us to reasonably expect that fear conditioning modulates such rapid short-lasting neural responses. For TANOVAs, it has been recommended that global duration statistics (indicating "overall" significance) should be treated as "overly conservative in light of pre-existing knowledge about the functional correlates of certain analysis periods" (Habermann et al., 2018). With regard to early-latency ERP modulations (as the present effect), which tend to be of a shorter duration, global duration statistics are particularly conservative. During 33–60 ms, the TANOVA showed a significant difference between CS+ and CS- topographies that did not exceed the more conservative overall duration threshold of 56.64 ms.

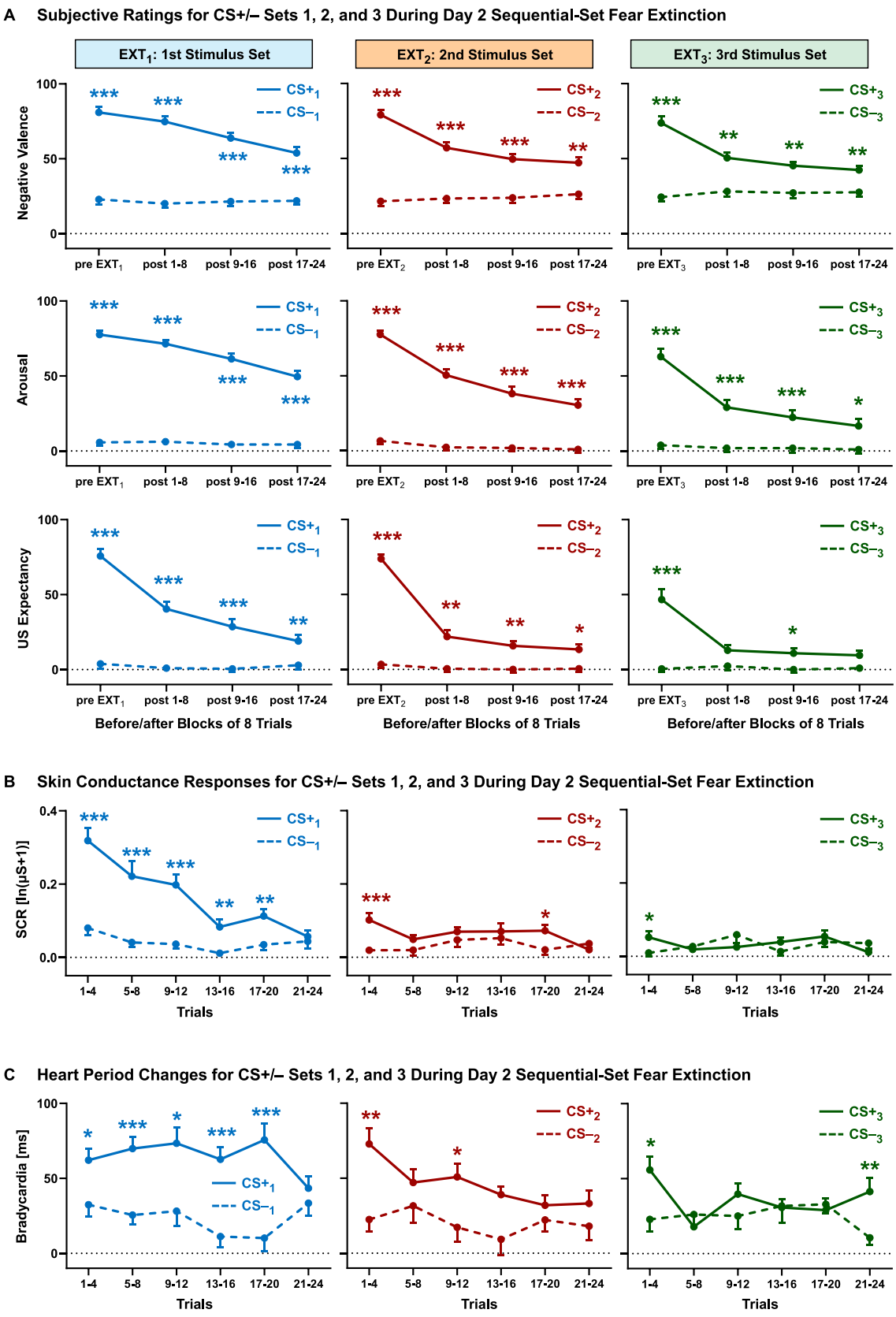

Fig. 3. Subjective and peripheral physiological (electrodermal activity and electrocardiogram) correlates of day 2 sequential-set fear extinction. Conditioned responses are plotted for (A) ratings of CS-associated valence (0 = "very good", 100 = "very bad"), arousal (0 = "not aroused at all", 100 = "extremely aroused"), and US expectancy (0% = "US will definitely not coterminate", 100% = "US will definitely coterminate"), (B) CS-evoked SCR amplitudes, and (C) CS-related heart period slowing (fear bradycardia). Conditioned responses are shown separately for the three CS sets (e.g., $CS+_1$ versus $CS-_1$). Subjective ratings were collected *before* each extinction training phase and *after* blocks of eight trials each. Blocks for peripheral physiological data consisted of four trials each. Line charts display mean values ± within-subject standard errors of the mean (*SEM*, O'Brien and Cousineau, 2014). $^{***}p \leq .001$, $^{**}p \leq .01$, $^{*}p \leq .05$.

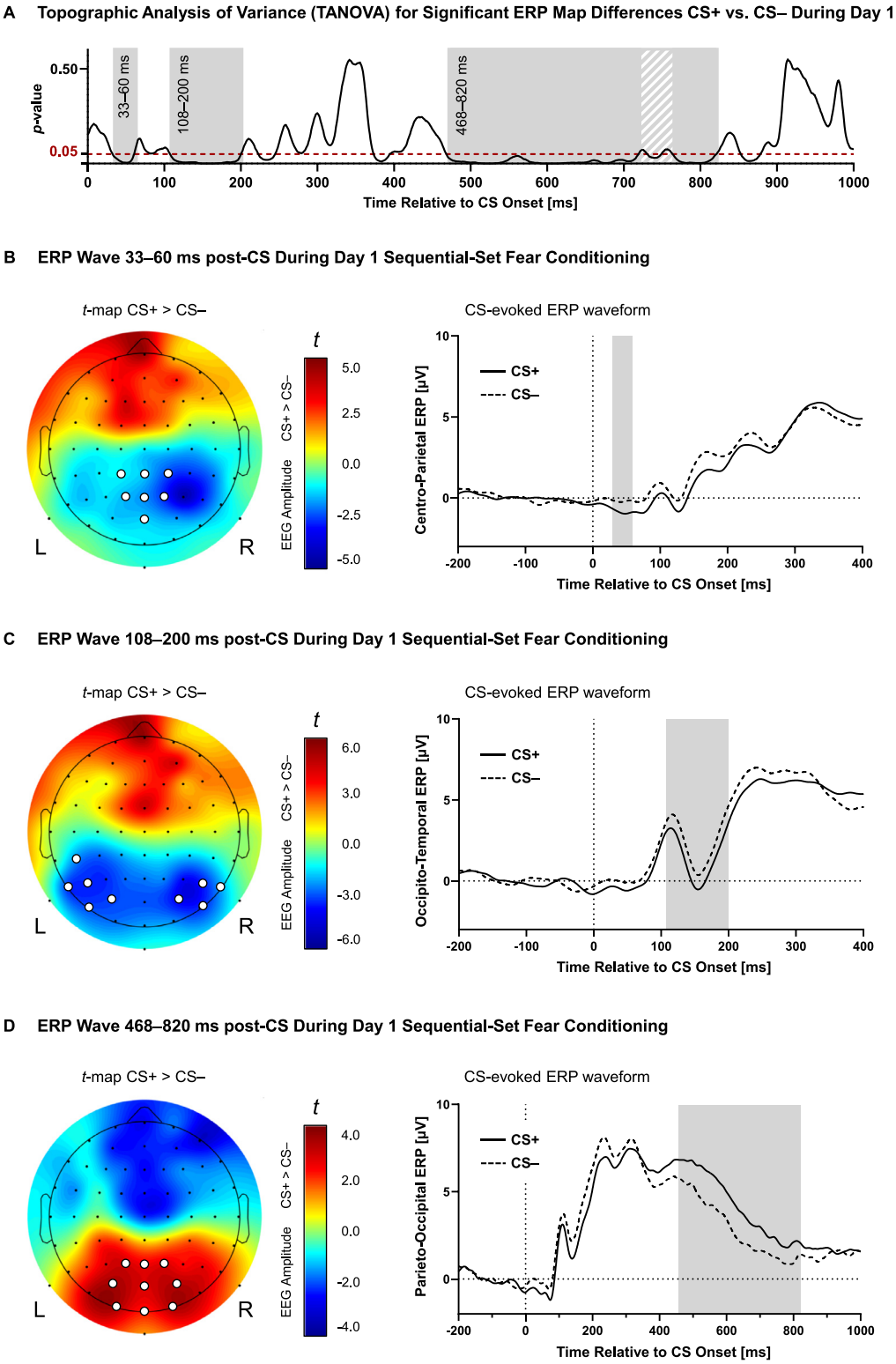

Fig. 4. Event-related potential (ERP) responses evoked by CS+ compared with CS- during day 1 sequential-set fear conditioning. (A) The topographic analysis of variance (TANOVA) indicated that topographic maps were significantly different for CS+ compared with CS- during the 33–60 ms, 108–200 ms, and 468–820 ms periods (i.e., *ps* ≤ .05, gray-shaded area). The last time window was interrupted by a short period (719–730 ms) with .05 ≤ *p* ≤ .08 (shaded in gray and white). (B) During 33–60 ms, the ERP amplitude was significantly more negative for CS+ compared with CS- at centro-parietal electrode sites (left panel). To visualize ERP waveforms (right panel), the electrode sites CP1, CPz, CP2, P1, Pz, P2, and POz were averaged (channels are shown as white dots in the *t*-map). (C) During 108–200 ms, the ERP amplitude was significantly more negative at occipito-temporal channels for CS+ versus CS-. The ANOVA on occipito-temporal ERP amplitudes yielded a significant *Contingency* x *Channel* interaction, and significantly more negative amplitudes for CS+ compared with CS- occurred at CP5, P7, P5, PO7, and PO3 over the left hemisphere, as well as at P8, P6, PO8, and PO4 over the right hemisphere (channels are shown as white dots in the *t*-map). To visualize ERP waveforms (right panel), electrodes with significant effects were averaged. (D) During 468–820 ms, the ERP amplitude was significantly more positive at parieto-occipital channels for CS+ versus CS- (left panel). To visualize ERP waveforms (right panel), the electrode sites P1, Pz, P2, PO3, POz, PO4, O1, Oz, and O2 were averaged (channels are shown as white dots in the *t*-map, significant effects could be confirmed at all electrode sites). The gray-shaded areas in panels B, C, and D indicate the measurement windows for ERP amplitudes. "L" = left hemisphere, "R" = right hemisphere. Note: A schematic illustration of the EEG montage with electrode labels is available at https://doi.org/10.5281/zenodo.4294603.

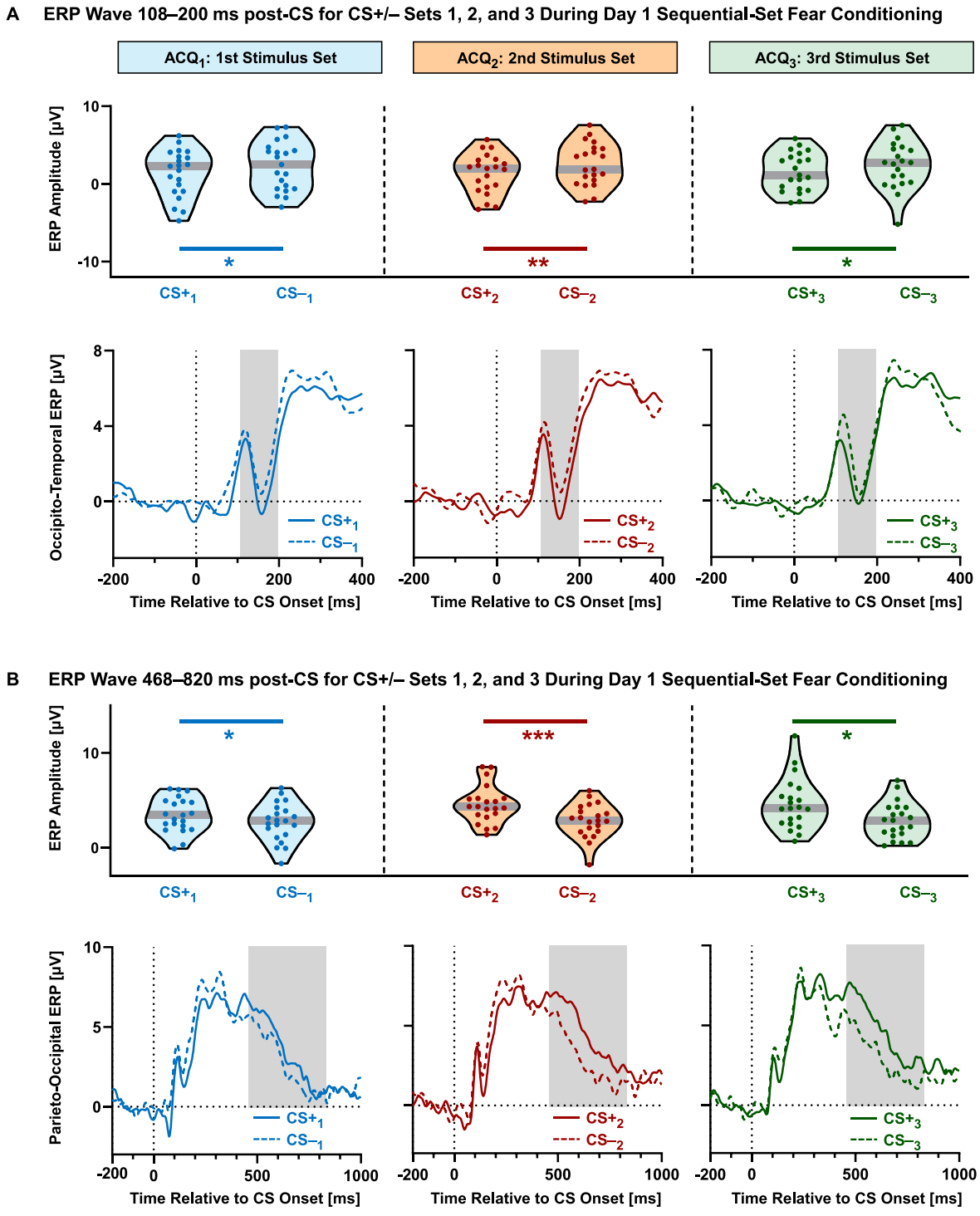

Fig. 5. Event-related potential (ERP) responses evoked by CS+ compared with CS- during day 1 sequential-set fear conditioning were comparable across the three stimulus sets. (A) The ERP amplitude during 108–200 ms after CS onset was significantly more negative for CS+ compared with CS- in all three CS sets. The *Contingency* x *Set* x *Channel* x *Hemisphere* ANOVA yielded a significant *Contingency* x *Channel* interaction, and the ERP amplitudes were significantly more negative for CS+ compared with CS- at CP5, P7, P5, PO7, and PO3 over the left hemisphere, as well as at P8, P6, PO8, and PO4 over the right hemisphere. To visualize ERP waveforms during this period, electrodes with significant effects were averaged. (B) The ERP amplitude during 468–820 ms time-locked to the CS onset was significantly more positive for CS+ compared with CS- in all three CS sets. To visualize ERP waveforms, the parieto-occipital electrode sites P1, Pz, P2, PO3, POz, PO4, O1, Oz, and O2 were averaged (significant effects could be confirmed at all electrode sites). In the upper panels, violin plots display the frequency distribution of the ERP data. Individual data points are superimposed on the violin plot, and the median is displayed as a grey horizontal line. In the lower panels, the time series of CS-evoked changes in voltage (relative to baseline) are shown. Gray-shaded areas indicate time windows for statistical analyses. $^{***}p \leq .001$, $^{**}p \leq .01$, $^{*}p \leq .05$.

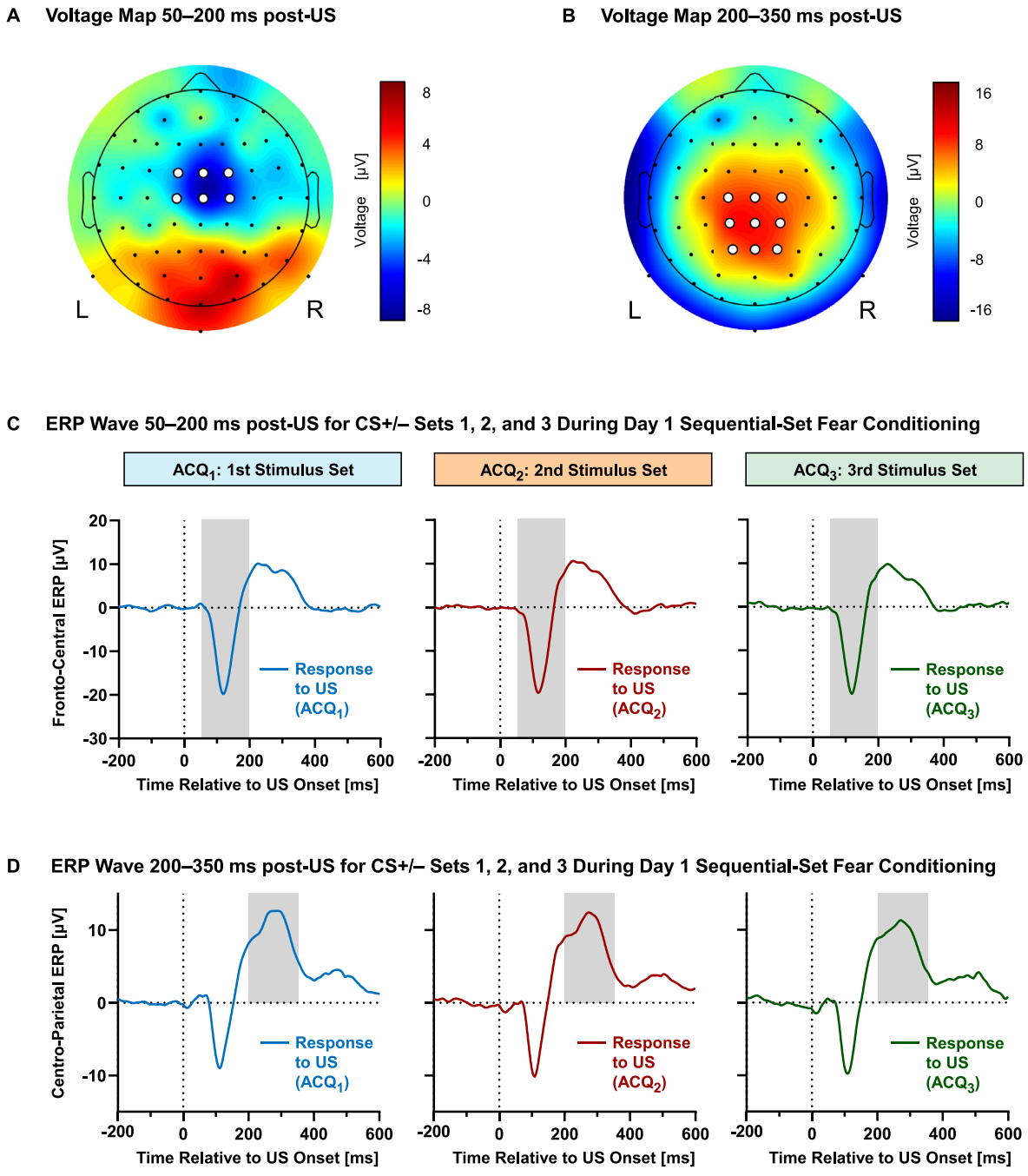

Fig. 6. Event-related potential (ERP) responses evoked by the US during day 1 sequential-set fear conditioning were comparable across the three acquisition training phases for CS set 1, set 2, and set 3. The US was associated (A) with a large fronto-central negative deflection from 50 to 200 ms, followed by a (B) centro-parietal positive deflection from 200 to 350 ms after stimulus onset (gray-shaded areas). (C) To visualize ERP waveforms during the 50–200 ms period, the electrode sites FC1, FCz, FC2, C1, Cz, and C2 were averaged (channels are shown as white dots in the voltage map). (D) To visualize ERP waveforms during the 200–350 ms period, the electrode sites C1, Cz, C2, CP1, CPz, CP2, P1, Pz, and P2 were averaged (channels are shown as white dots in the voltage map). "L" = left hemisphere, "R" = right hemisphere. Note: A schematic illustration of the EEG montage with electrode labels is available at https://doi.org/10.5281/zenodo.4294603.

showed a significant main effect of *Channel* ($F(5,100) = 8.27$, $p < .001$). Effects were most substantial at midline channels, and extended mainly to channels over the right hemisphere (see Fig. 6A), consistent with previous research indicating enhanced amplitudes during this period over channels *contralateral* to the hand receiving electric shocks (Wang et al., 2014). Conversely, during the 200–350 ms period, we observed a reliable positive deflection (see Fig. 6D) at centro-parietal channels C1, Cz, C2, CP1, CPz, CP2, P1, Pz, and P2. Unconditioned responses during both time windows did not habituate and were similar across CS sets (no repeated-measures effects of *Set*, all $ps \geq .179$)

Collectively, ERP analyses revealed that fear conditioning was accompanied by enhanced EEG amplitudes for CS+ compared with CS- during three distinct time windows. Conditioned responses were similar across all three CS+/CS- sets. To calculate effect sizes for ANOVA main effects, the mean values for CS+ and CS- were computed (averaged across other ANOVA factors). According to Cohen's (1988, 1992) benchmark, participants learned a *large* electrocortical fear response (CS+ versus CS-) for the 108–200 ms ($d = 1.11$) and 468–820 ms ($d = 0.85$) time windows, whereas they acquired a *medium to large* effect for the 33–60 ms period ($d = 0.47$).

3.4. EEG: ERP components during fear extinction

For day 2 fear extinction, the TANOVA (see Supplementary Material D) indicated significant differences between ERP maps for CS+ and CS- in the time window from 460 to 730 ms after CS onset (averaged across the significant time window: TANOVA $p < .001$). An additional TANOVA on the amplitude-normalized maps confirmed that, similar to effects during the acquisition stage, different intracranial brain generators were involved (see Supplementary Fig. S4). In earlier time windows, the TANOVA did not reach significance and topographies for CS+ and CS- were comparable.

460–730 ms post-CS. This time window is very similar to the late-latency period we observed during day 1 fear conditioning. The topography of the grand-grand average ERP converged with results from day 1, and we observed a sustained positive deflection at parieto-occipital electrode sites. Emphasizing the robustness and validity of the conditioned fear response, the TANOVA for the extinction stage suggested that late-latency conditioning effects, which have already been reported during day 1, remained significant 24 h later. We computed a *Contingency* x *Set* x *Channel* x *Time* ANOVA at parieto-occipital channels P1, Pz, P2, PO3, POz, PO4, O1, Oz, and O2. This analysis confirmed that CS+ evoked a larger positivity compared with CS- (*Contingency* main effect, $F(1,20) = 22.50$, $p < .001$). As expected, a trendwise *Contingency* x *Time* interaction ($F(2,40) = 2.97$, $p = .063$) indicated that the differential fear response declined from early to late extinction training, a phenomenon that supports the formation of a new extinction memory trace. For all individual EEG channels included in the ANOVA, paired-samples *t*-tests confirmed significant effects. Importantly, these late ERP effects during extinction were comparable across CS sets, and follow-up ANOVAs for individual sets confirmed significant *Contingency* main effects for CS set 1 ($F(1,20) = 16.36$, $p = .001$), set 2 ($F(1,20) = 11.33$, $p = .003$), and set 3 ($F(1,20) = 14.33$, $p = .001$). Accordingly, slow-wave conditioning effects remained significant 24 h after fear conditioning.

3.5. EEG: learning dynamics of ERP effects as revealed by block-wise analyses after averaging across CS sets

Due to the relatively low signal-to-noise ratio of EEG signals, averaging across a large number of trials is necessary to detect ERP signatures of fear conditioning (Miskovic and Keil, 2012; Steinberg et al., 2013; Huffmeijer et al., 2014). However, such aggregations will wash out temporal changes in neural responding over time (Ferreira de Sá et al., 2019). In fact, given that the subjective expectancy regarding the CS-US contingency is changing throughout learning, we assume that neural fear responses are not stable across all trials. Instead, differential conditioned responses rise from early to late conditioning and decline from early to late extinction learning. This fact has often been ignored in conventional EEG studies (Miskovic and Keil, 2012). In contrast, *sequential-set* conditioning allows us to average across CS sets at specific time points in the conditioning stage, creating the possibility to tap into more transient neurophysiological processes and to detect *temporal changes* during learning with a sufficient signal-to-noise ratio.

To detect changes over time, the acquisition training phases were split into three sub-blocks of five trials each. In a similar way, the extinction training phases were split into six sub-blocks of four trials each. To control for EEG responses before conditioning, the four pre-acquisition trials were also averaged. Despite averaging only a small number of trials within each stimulus set (e.g., five trials during conditioning), averaging across all three CS sets triples the number of trials. As reported above, the acquisition ANOVA for the 108–200 ms period yielded a significant *Contingency* x *Channel* interaction, while statistical analyses for the 33–60 ms and 468–820 ms periods showed comparable effects for all channels included in the ANOVAs. To analyze temporal changes during learning, electrodes with significant effects were averaged. Importantly, these analyses on learning dynamics of EEG effects revealed a linear growth of electrocortical fear responses from early to late conditioning.

Differential EEG responses during 33–60 ms after CS onset increased throughout fear acquisition trials, as indicated in Fig. 7A. Specifically, a polynomial trend analysis showed that differential fear responses followed a linear growth curve (linear trend for the *Contingency* x *Sub-Block* interaction: $F(1,20) = 7.33$, $p = .014$). There was no difference during pre-acquisition ($t(20) = 0.90$, $p = .381$), but ERP responses were significantly larger (i.e., more negative) during the last five acquisition training trials ($t(20) = -3.70$, $p = .001$). Likewise, effect sizes increased step by step (see blue bars in Fig. 7A), and we observed a large effect ($d = 0.81$) toward the end of conditioning.

There was a similar pattern 108–200 ms after CS onset (see Fig. 7B). Differential fear responses increased from early to late conditioning (linear trend for the *Contingency* x *Sub-Block* interaction: $F(1,20) = 9.60$, $p = .006$). During the pre-acquisition trials, there was no difference between ERP responses to CS+ and CS- ($t(20) = 1.60$, $p = .126$). Conversely, CS+ compared with CS- evoked a significantly larger negativity during the last four acquisition trials ($t(20) = -3.12$, $p = .005$). Effect sizes showed a sharp rise during the first five acquisition training trials and reached a plateau with notably smaller subsequent changes (see blue bars in Fig. 7B).

Finally, conditioned fear responses during the 468–820 ms post-CS period (see Fig. 7C and E) also followed a linear learning curve, and polynomial analyses confirmed a linear trend for the *Contingency* x *Sub-Block* interaction ($F(1,20) = 21.64$, $p < .001$). While CS-evoked ERPs were comparable during the pre-acquisition trials ($t(20) = -1.08$, $p = .294$), CS+ versus CS- led to a significantly stronger positivity toward the end of acquisition training ($t(20) = 3.68$, $p = .001$). Effect sizes constantly increased during fear conditioning (see blue bars in Fig. 7C).

Twenty-four hours later, no effects could be detected for short- and mid-latency ERPs (see Supplementary Material E), suggesting that sensory processing was similar for CS+ and CS-. However, differential fear responses in the late-latency period from 460 to 730 ms (see Fig. 7D and F) gradually diminished from early to late extinction learning (linear trend for the *Contingency* x *Sub-Block* interaction: $F(1,20) = 8.80$, $p = .008$). Specifically, CS+ compared with CS- evoked a significantly larger positivity during the first four extinction training trials ($t(20) = 4.64$, $p < .001$). Differential fear responses vanished toward the end of extinction training ($t(20) = 0.93$, $p = .362$). Similarly, effect sizes successively declined from trial to trial during extinction training (see blue bars in Fig. 7D).

4. Discussion

Fear conditioning and extinction describe learning processes during which fear responses *increase* and *decrease* over time, respectively. The overarching goal of this study was to reconstruct the learning curves of neural processes during fear conditioning and extinction in humans. To date, several studies have investigated ERPs during fear conditioning (Miskovic and Keil, 2012), but little is known about how electrocortical signatures gradually evolve from trial to trial. The relatively poor signal-to-noise ratio of EEG recordings requires averaging across a high number of trials, a factor that impedes the analysis of fear learning from one moment to another (Steinberg et al., 2013; Huffmeijer et al., 2014). However, the informational value of CS+ and CS-, which is critical for learning, is changing during learning (Rescorla and Wagner, 1972; Tzovara et al., 2018) because the associative strength between CS+ and the US is gradually increasing (conditioning) or decreasing (extinction). Neurophysiological processes that are responsible for the initial acquisition of CS-US contingencies show a fast habituation pattern over time (Yin et al., 2018). Thus, neural responses that are specific for the formation of fear memories are particularly pronounced during early learning phases (Büchel et al., 1998; LaBar et al., 1998). Accordingly, neurophysiological indices of fear are supposed to change across trials due to learning (i.e., due to changes in associative strength) and habituation (i.e., due to repeated stimulation). A suitable paradigm that allows one to investigate neural dynamics of fear learning has been missing so far.

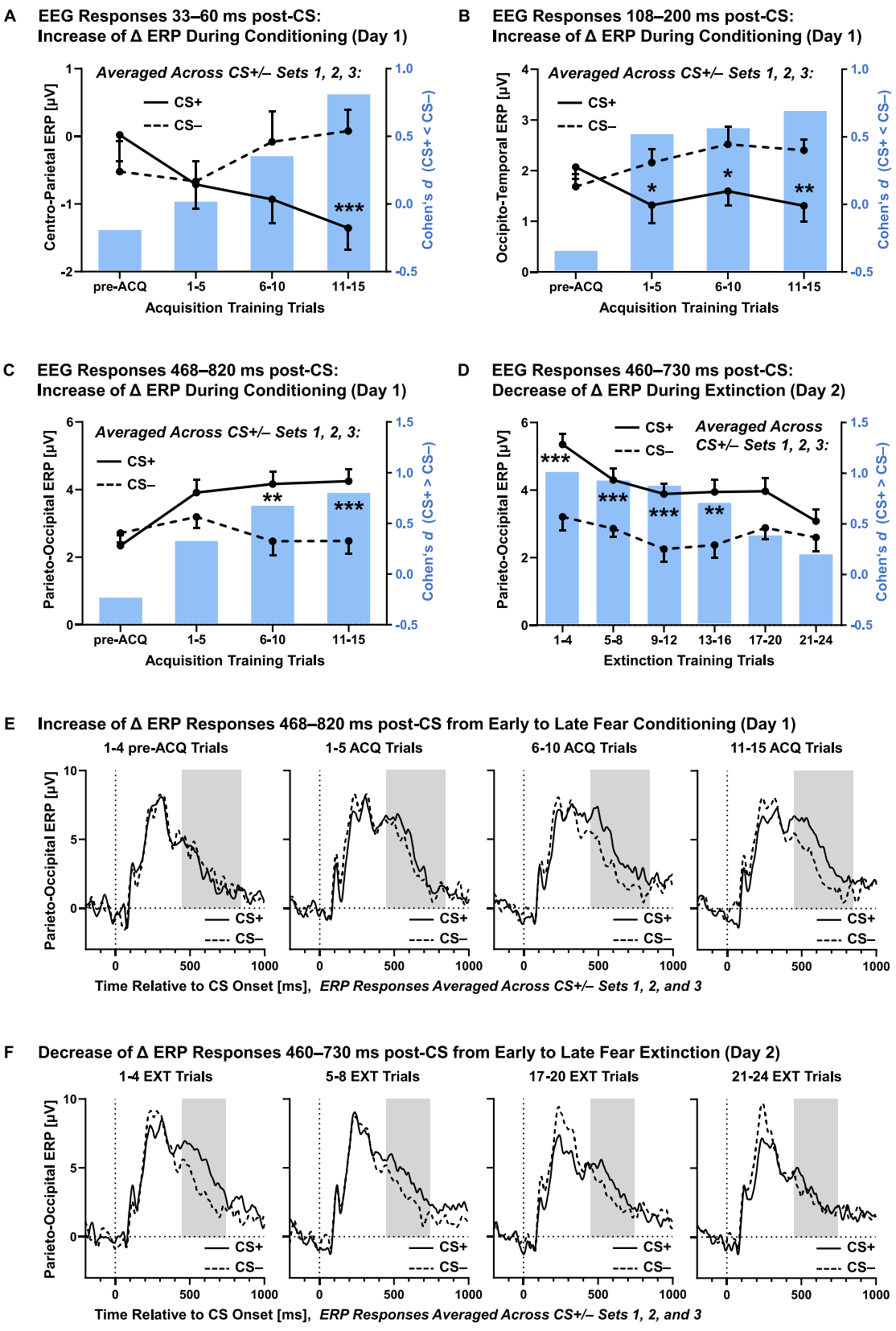

Fig. 7. To detect changes over time, the acquisition and extinction training phases were split into smaller sub-blocks of five (acquisition) or four (pre-acquisition and extinction) trials each. Averaging across trials from all three conditioned stimulus (CS+/CS-) sets allows studying the increase and decrease of Δ ERPs (CS+ versus CS-) during fear conditioning and extinction, respectively. Conditioned EEG responses during the (A) 33–60 ms, (B) 108–200 ms, and (C, E) 468–820 ms periods increased from early to late fear conditioning (day 1). Conversely, conditioned responses during (D, F) 460–730 ms decreased from early to late extinction (day 2). Line charts (A–D) show the mean voltage for CS+ and CS- for each sub-block (± within-subject *SEM*, O'Brien and Cousineau, 2014). Blue bars indicate how effect sizes (Cohen's *d*, plotted on the right y-axis) for conditioned electrocortical responses increased during fear conditioning (A–C) and decreased during fear extinction (D). $^{***}p \leq .001$, $^{**}p \leq .01$, $^{*}p \leq .05$.

Here, we fill this gap by providing evidence about transient changes in EEG responses during fear conditioning and extinction using a new *sequential-set* fear conditioning paradigm. To minimize attenuation of fear responses across trials, fear conditioning and extinction consist of three successive phases. For each phase, a novel CS+/CS- set is used. Importantly, changes in neural responding between smaller subsets of trials emerge after averaging across CS sets.

During fear conditioning, all reported measures pointed toward a similarly strong conditioned response for CS sets 1, 2, and 3. Consistent with prior research (Bradley et al., 2005; Castegnetti et al., 2016; Marin et al., 2020), CS+ versus CS- evoked elevated skin conductance responses and relative fear bradycardia, reflecting heightened physiological arousal and vigilance in anticipation of the US (Davis and Lang, 2003; Löw et al., 2015). Peripheral physiological responses to the CS+ may thus be interpreted as an indicator that on-going behavior was interrupted and attention was oriented toward the threat cues (Blanchard et al., 2011), which can be critical for survival (Mobbs et al., 2015). On the subjective level, CS+ compared with CS- was associated with higher ratings of negative valence, arousal, and US expectancy. Concerning EEG data, we did not have any *a priori* constraints and applied a *data-driven* approach to identify ERP components that are modulated by fear learning. Demonstrating the suitability of our paradigm to assess EEG responses to threat, we captured short-, mid-, and long-latency electrocortical processes. Specifically, CS+ compared with CS- elicited elevated ERP amplitudes during the 33–60 ms, 108–200 ms, and 468–820 ms periods. Our results suggest preferential and facilitated processing of aversive cues during various stages. Consistent with the conceptualization of a "threat sensitization" hypothesis (Bublatzky and Schupp, 2012), fear conditioning seems to prioritize neural transmission and enhance selective attention toward signals of danger. Notably, after averaging across CS sets, we were able to reconstruct learning curves for ERPs in these periods.

It is important to point out that relevant time windows for ERP analyses were derived from a data-driven approach. In contrast, ERP *components* are usually defined based on distinct electrophysiological properties (latency, polarity, amplitude, topography). ERP components are often conceptualized as peaks in the observed scalp waveform. However, this practice can be problematic, as underlying "latent" components may differ (Luck, 2014). Underlying components are considered to be neural processes which sum together and produce an ERP wave that can be measured with electrodes on the scalp (Luck and Kappenman, 2019). Though, it is important to remember that multiple underlying components mix in the scalp ERP waveform, which are difficult to disentangle. If an ERP component is quantified based on a pre-defined measurement window, it is most likely that EEG activity during this period is related to multiple underlying "latent" components. In addition, important effects may be missed if the analysis is limited to specific periods. Here, we circumvented this problem and used a data-driven approach to isolate time windows that are relevant for fear conditioning and extinction. Specifically, we computed a TANOVA and explored during which periods ERPs evoked by the CS+ and CS- differed in response strength and topography. Compared with "traditional" ERP analyses, this approach is much more flexible and powerful, as all electrodes and time frames are considered. There is no simple or one-to-one mapping between TANOVA-derived periods and "traditionally" defined ERP waves. Nevertheless, effects may overlap partly. In order to link our findings with the previous fear conditioning literature, it is helpful to speculate which ERP components might be involved during the time windows reported in the present study. Sketching out relationships between specific ERP periods and different brain functions allows to draw inference about how threatening cues guide attention and processing speed (Bublatzky et al., 2010; Bublatzky and Schupp, 2012; MacNamara et al., 2013; Klumpp and Shankman, 2018).

First, we found a more negative ERP amplitude to CS+ versus CS- as early as 33–60 ms after CS onset, suggesting rapid detection of fear-conditioned stimuli and privileged signal transmission during the earliest processing stages. Some previous EEG studies demonstrated that fear conditioning can modulate already early processing in visual cortices (Stolarova et al., 2006; Hintze et al., 2014; Mueller and Pizzagalli, 2016; Thigpen et al., 2017). Mirroring the results of our data-driven approach, amplified neural responses to visual fear-conditioned stimuli have been reported as early as 30–60 ms (Morel et al., 2012), 41–55 ms (Mueller and Pizzagalli, 2016), and 50–80 ms (Steinberg et al., 2012, 2013). Subcortical brain regions may be closely linked with perceptual areas (Freese and Amaral, 2005; Chen et al., 2009; Pourtois et al., 2013) and initially gate threat processing in visual regions (Vuilleumier et al., 2004; Rotshtein et al., 2010). Research in macaque monkeys revealed a substantial modulatory control of amygdaloid projections over processing in sensory pathways (Amaral et al., 2003), which may thereby boost early brain responses to emotional information (Vuilleumier, 2009). Over the course of learning, plastic changes in primary visual cortex neurons may further lead to a facilitated perception of threat stimuli (Keil et al., 2007; McTeague et al., 2015; Thigpen et al., 2017). Sparsification of neural representations as well as enhanced synaptic efficiency may explain this successive shift in processing toward neurons with shorter response latencies (Stegmann et al., 2020; Wieser and Keil, 2020). This interpretation is in line with the idea that fear memory formation initially involves a widespread neural network, which may be sharpened across learning trials to involve more specialized neurons in sensory regions (Moratti et al., 2006; Miskovic and Keil, 2012; Thigpen et al., 2017). Crucially, the learning curve of the 33–60 ms effects in our data closely mirrors this hypothesis of subsequent visual cortex plasticity: While there was no significant difference in ERP responses to CS+ and CS- during early conditioning, we observed a large differential fear response only during the last five conditioning trials. After repeated learning experiences, short-term plasticity may promote biased perception even in early regions of the visual hierarchy, which have not been considered to be sensitive for attentional modulations in traditional models (Gomez Gonzalez et al., 1994; Clark and Hillyard, 1996; Anderson, 2011). Linking this effect to "classical" ERP components is challenging. The C1 wave is considered to be one of the earliest visual ERP components and is thought to be generated mainly in the primary visual cortex (Jeffreys and Axford, 1972; Clark et al., 1994; Rauss et al., 2011). The C1 wave typically starts 40–60 ms and peaks 80–100 ms after stimulus onset (Luck, 2014). In the present study, we found an effect beginning at 33 ms already, which is earlier compared to genuine C1 waves described in the literature. However, animal studies report that the earliest visual response latencies in the macaque primary visual cortex start around 35 ms after stimulus onset (Lamme and Roelfsema, 2000). Using intracerebral ERP recordings in epileptic patients, Kirchner et al. (2009) demonstrated that sensory characteristics of visual stimuli can modulate neural activity during ultra-rapid latencies between 45 and 60 ms after stimulus onset. Furthermore, electromagnetic studies in humans suggest discriminative processing of face stimuli during very early latencies of 30–60 ms (Braeutigam et al., 2001) and 40–50 ms (Morel et al., 2009). Responses to non-face stimuli were weaker and less widespread (Braeutigam et al., 2001), suggesting that a fast detection of face stimuli has been of particular relevance in the evolutionary past (Kret and Gelder, 2012). Our findings emphasize that fear conditioning can boost the earliest stages of visual processing, which may either be related to subcortical projections or – during later trials – reflect an ultra-rapid feed-forward flow of sensory information (Pourtois et al., 2013; Thigpen et al., 2017).

Second, fear conditioning was associated with potentiated ERP amplitudes between 108 and 200 ms after CS onset, which may reflect privileged sensory processing of threat in extrastriate regions (Clark and Hillyard, 1996; Linkenkaer-Hansen et al., 1998). This is a relatively broad time window, which includes the typical latencies of the P1 and N170 ERP components (Desjardins and Segalowitz, 2013). During this period, CS+ versus CS- faces evoked more negative amplitudes at occipito-temporal channels, corresponding to the typical scalp distribution of these components (Eimer, 2011; Rossion and Jacques, 2012;

Luck, 2014). Remarkably, our results also demonstrate the temporal evolution of fear conditioning effects during the 108–200 ms period: We observed a significantly more negative ERP amplitude for CS+ compared with CS- already during the first five conditioning trials, while differential effects remained stable and slightly increased over the subsequent course of conditioning. Rotshtein et al. (2010) reported that amygdala damage leads to diminished ERPs for fearful faces during 100–150 ms post-stimulus. Considering the crucial role of the amygdalar circuits for fear conditioning (LeDoux, 2014; Janak and Tye, 2015), we can assume that this time window is of particular relevance for rapid threat processing already during early conditioning trials. With regard to the common ERP literature, our observation during the 108–200 ms period may be linked to a combination of attenuated (i.e., less positive) P1 amplitudes and magnified (i.e., more negative) N170 amplitudes. Effects related to these processes are difficult to disentangle with the current experimental design. Liu et al. (2012b) showed decreased P1 amplitudes after CS+ versus CS- for well-trained stimulus pairs. Consistent with the prediction error theory of attention during classical conditioning (Pearce and Hall, 1980), we may speculate that the required level of attention decreases as the US is fully predicted by the CS (Liu et al., 2012b). Changes in US expectancy seem to be critical for learning, especially during *early* fear conditioning trials (Wills, 2009). Supporting our interpretation, ERP and eye tracking studies suggest a correlation between differences in attention and the size of the previously produced prediction error (Wills et al., 2007; Wills, 2009). In addition, similar to our findings, Rigoulot et al. (2008) reported a P1 reduction for unpleasant compared with neutral pictures. Given that we used different face stimuli as CSs, it is important to keep in mind that the 108–200 ms period can also include activity which may be related to the N170 component. This component is particularly enhanced for faces (Eimer, 2011; Schweinberger, 2011; Rossion and Jacques, 2012) and involves face-selective generators (McKone and Robbins, 2011) from the fusiform gyrus (Gao et al., 2019). Larger (i.e., more negative) N170 amplitudes for CS+ compared with CS- may reflect heightened allocation of attentional resources to fear-conditioned faces, due to their high evolutionary significance (Kret and Gelder, 2012). While some studies negated an emotional modulation of the N170 complex (e.g., Eimer et al., 2003; Holmes et al., 2005), others reported larger amplitudes for fearful compared with neutral facial expressions (e.g., Blau et al., 2007; Schindler et al., 2019). Likewise, some electromagnetic fear conditioning studies have reported that faces or face-like stimuli that signal danger elicit changes in brain activity during the N170 period (Pizzagalli et al., 2003; Steinberg et al., 2012; Levita et al., 2015; Camfield et al., 2016; Mueller and Pizzagalli, 2016; Watters et al., 2018).

Third, at 468–820 ms from CS onset, CS+ compared with CS- elicited greater positivity at parieto-occipital EEG channels. The late latency and topographic distribution make us reasonably assume that effects during this period presumably reflect activity of the LPP (Schupp et al., 2006; Hajcak et al., 2018; Hajcak and Foti, 2020). High arousal and motivational salience of emotional stimuli consistently evoke amplified LPP responses, which can persist for hundreds of milliseconds (Schupp et al., 2006; Hajcak et al., 2018) and are generated in an extensive cortical and subcortical network (Liu et al., 2012a). A body of fear conditioning studies (Panitz et al., 2015, 2018; Pastor et al., 2015; Bacigalupo and Luck, 2018; Seligowski et al., 2018; Ferreira de Sá et al., 2019; Pavlov and Kotchoubey, 2019; Stolz et al., 2019) has provided evidence that CS+ evokes larger LPP amplitudes than CS-. Amplified LPP responses for the CS+ can be interpreted as an indicator of stimulus significance (Hajcak and Foti, 2020), reflecting elaborative processing and the activation of a cortico-limbic defensive system (Bradley, 2009). In terms of “motivated attention”, emotionally arousing stimuli activate motivational circuits in the brain which are related to survival behavior (e.g., escape, attack) and require a sustained allocation of attentional resources (Lang et al., 1997; Schupp et al., 2004; Pastor et al., 2008). Extending previous findings, we observed a stepwise increase of slow-wave fear responses during conditioning. Our data indicate that effect sizes accumulated from trial to trial, and a large fear response was acquired toward the end of conditioning. Together, these findings suggest that sustained attention and elaborative processing of the threat-predicting CS+ (Cuthbert et al., 2000; Nelson et al., 2015b; Weinberg et al., 2015) progressively gained during learning. In contrast to mid-latency responses, ERPs from 468 to 820 ms showed a slower increase and were particularly pronounced during late conditioning trials, which may represent functional differences in attentional processes. During later conditioning trials, the uncertainty about the CS-US contingencies gets gradually reduced and the US becomes reliably predicted by the CS+. Thus, the danger is getting more imminent (Davis and Lang, 2003; Lang and Bradley, 2013; Löw et al., 2015), which requires the preparation of defensive threat reactions (Roelofs, 2017). With growing awareness about the CS-US contingency, motivational top-down factors (e.g., emotional evaluation, cognitive reappraisal and regulation strategies, active searching for threat cues) may become more and more important (Olofsson et al., 2008; Hajcak et al., 2010; Mohanty and Sussman, 2013; Myruski et al., 2019).

As discussed above, fear conditioning was accompanied by relatively more negative ERP activity to the CS+ compared with CS- during short- (33–60 ms) and mid-latency (108–200 ms) periods. Moreover, during the late-latency period (468–820 ms), this effect basically swaps, and we observed a more positive amplitude for CS+ versus CS-. Considering this intriguing dynamic across the three time windows, the late-latency effect (468–820 ms) was evident as a sustained positivity across parieto-occipital sites and can thus be reliably interpreted as an indicator of enhanced stimulus significance for the CS+, accompanied by sustained allocation of attentional resources (Hajcak and Foti, 2020). However, the interpretation of the polarity of effects during the short-latency period (33–60 ms) is more challenging. Due to the retinotopic organization of the striate cortex, ERPs within the first 100 ms after stimulus onset can either appear as a negative or positive voltage deflection, depending on whether the stimulus was presented in the upper or lower visual field, respectively (Clark et al., 1994). Here, we observed an early negativity at centro-parietal channels for the CS+, which was almost absent for the CS-. Thus, we believe that this effect represents a larger negativity for CS+ compared with CS-, which can be interpreted as *elevated* sensory processing. However, we cannot fully exclude that this effect might be driven by a reduced positivity for the CS+, which would indicate *attenuated* sensory processing. This alternative explanation could be ruled out in future studies if the location of the CS+ and CS- would explicitly be varied between the upper and lower visual field. During the 108–200 ms period, the voltage was more negative for CS+ versus CS-. This time window comprises a relatively long period, which includes neural processes that may be linked to the P1 and N170 components. Thus, this effect could be related to a reduced positive deflection, to a larger negative deflection, or to a combination of both. On the one hand, as discussed above, an attenuated positivity could indicate that less attention is required if the US is reliably predicted by the CS+ as fear conditioning proceeds, which would be consistent with the P1 literature (Liu et al., 2012b). On the other hand, following the N170 literature, a larger negativity could reflect heightened attentional engagement with fear-conditioned faces (Eimer, 2000, 2018; Schweinberger, 2011). To disentangle both processes, future studies should assess whether effects can be replicated with non-face CSs, which would reduce the influence of processes that are related to the face-sensitive N170 component.

For day 2, the TANOVA on the first extinction training block (i.e., the first 8 trials) revealed a larger positivity for CS+ compared with CS- at parieto-occipital channels between 460 and 730 ms after CS onset. Conditioned responses were similar for extinction phases 1, 2, and 3, underlining the robustness of this slow-wave fear response. The latency and topography of effects during this period converge with the LPP-related time window that we observed during fear conditioning on day 1. As expected, LPP-related effects faded over the course of extinction learning. The decline of this late-latency fear response indicates that less allocation of attentional resources is required when the CS+ faces no longer

predict an aversive outcome. Violating the US expectancy (Craske et al., 2018) seems to be critical for the formation of a new extinction memory trace (Bouton, 2017), which alters the predictive value (Myers and Davis, 2007) and stimulus significance (Hajcak and Foti, 2020) of the CS+. Reduced LPP responses during fear extinction may be related to top-down inhibitory signaling (Pourtois et al., 2013) from prefrontal areas (Adhikari et al., 2015; Jayachandran et al., 2019; Marek et al., 2019), which are crucially involved in extinction learning (Milad and Quirk, 2012). The decrease of late-latency ERP responses was accompanied by diminished electrodermal and cardiac fear indices. Altogether, we assume that extinction learning was presumably associated with a reduction in motivated attention toward the CS+ (Lang et al., 1997; Schupp et al., 2004; Pastor et al., 2008).

Previous fear conditioning studies were primarily interested in amplitude differences between ERPs evoked by CS+ and CS-. Conversely, topographic differences between conditions have often been ignored in EEG research (Michel and Murray, 2012). Here, we used a data-driven TANOVA approach which captures ERP effects that may be related to differences in both amplitude strength (i.e., amount of simultaneously active sources) *and* topography (i.e., location/orientation of active sources). Additional analyses on differences between the amplitude-normalized maps revealed that our ERP effects seem to be partially related to different generator configurations. Notably, Murray et al. (2008) hypothesized that stimuli of negative emotional valence may be processed through a more efficient neural circuit, which would imply the contribution of (at least partially) different generators. This interpretation is consistent with our findings, suggesting that fast and prioritized signaling for fear-conditioned stimuli may, to some extent, involve segregated neural pathways (LeDoux, 1995, 2000).

Because of their high preparedness for fear conditioning, we used faces as CSs (Lissek et al., 2005), which seem to be processed in a rapid and automatic fashion in the human brain (Palermo and Rhodes, 2007; Tamietto and Gelder, 2010). Due to their evolutionary significance, a large amount of studies investigated ERPs to face stimuli in order to uncover attentional processes. In a recent review, Schindler and Bublatzky (2020) synthesize findings on emotional face processing, and point out that the influence of attention and emotion on face perception highly depends on the visual processing stage. The most consistent finding seems to be that attention to fearful faces leads to enhanced P3/LPP amplitudes (Schindler and Bublatzky, 2020), which may be explained by a larger impact of controlled attention (Hajcak et al., 2009) on later processing stages, especially toward potential danger (Schindler et al., 2020). This observation is complemented by our findings, as we detect late-latency fear responses during both fear conditioning and fear extinction stages. In contrast, short- (33–60 ms) and mid-latency (108–200 ms) ERP modulations emerged only during fear conditioning, but not during fear extinction. Mueller and Pizzagalli (2016) reported that remotely fear-conditioned faces can modulate rapid (< 80 ms) processing in visual brain regions even one year after acquisition, suggesting that conditioning effects might have been less stable in the present study. Furthermore, early ERP responses could depend more heavily on the threatening nature of the experimental context (Gelder et al., 2006; Muench et al., 2016), which may differ between conditioning and extinction stages. Moreover, transient and earlier brain processes may primarily be involved in the *acquisition* of emotional memories (Ferreira de Sá et al., 2019), which requires fast adaptation to threat. We assume that fear conditioning recruits a sensory-vigilance network, which is governed by the amygdala and fast projections to sensory cortices (Davis and Whalen, 2001; Sabatinelli et al., 2009; Shackman et al., 2011). Conversely, extinction learning seems to be mediated by top-down controlled influences from the prefrontal cortex (Milad and Quirk, 2012; Adhikari et al., 2015; Marek et al., 2019), which may affect rather late processing stages (Pourtois et al., 2013).

Taken together, we successfully demonstrated that sequential-set conditioning prevents habituation to the CSs, and allows to uncover the learning dynamics of perceptual and attentional processes. In addition to CS-evoked responses, we also assessed unconditioned responses. In a previous study we demonstrated that fear conditioning can be dramatically impaired if the US intensity does not remain high enough throughout acquisition trials (Sperl et al., 2016). To overcome this problem, the electrotactile US was applied in a relatively high shock intensity compared with the majority of fear conditioning studies (Sehlmeyer et al., 2009; Lonsdorf et al., 2017). As intended, peripheral and central physiological responses to the US resisted habituation. We observed a similarly strong unconditioned response during the acquisition trainings of CS set 1, set 2, and set 3. On the peripheral physiological level, the US evoked robust SCRs and cardiac acceleration, supporting fight-or-flight behavior. Replicating previous findings, we demonstrated that the US evoked an accelerative response (Ginsberg and Thysell, 1966; Lipp and Vaitl, 1990; Vila et al., 2007; Mueller et al., 2019), while the CS+ (as discussed above) was associated with relative heart rate deceleration. At first glance, this divergence between autonomic unconditioned and conditioned responses may seem paradoxical. However, the relative dominance of sympathetically driven acceleration and parasympathetically dominated deceleration gives critical insight into the functional meaning of attentional changes during different stages of threat proximity (Obrist, 1976; Davis and Lang, 2003; Löw et al., 2015): Anticipation of threat (decelerative responses to the CS) requires allocation of attentional resources, heightened vigilance, and facilitated sensory intake (“attentive freezing”). The goal of these attentional mechanisms is to mobilize and prepare the organism for later action responses (Lang and Bradley, 2010; Roelofs, 2017). In contrast, when the threat is most imminent (accelerative responses to the US), increased systemic activation and active defensive behavior are required, culminating in overt fight-or-flight responses (Davis and Lang, 2003; Lang and Bradley, 2013; Löw et al., 2015).

On the neural level, the US evoked a sharp fronto-central negative deflection from 50 to 200 ms, followed by a broader positive deflection from 200 to 350 ms which was maximal at rather centro-parietal electrode sites. These spatiotemporal characteristics match with previous studies investigating somatosensory ERPs to electrotactile stimuli (Miltner et al., 1989; Yamaguchi and Knight, 1991; Deguchi et al., 1996; Christmann et al., 2007; Kenntner-Mabiala et al., 2008; Wang et al., 2014; Nelson et al., 2015a; Wang and Tian, 2018). Both components seem to be sensitive to attentional modulations (Zaslansky et al., 1996; Eimer and Forster, 2003). The early negative complex is assumed to reflect mainly somatosensory processing of the aversive stimulus (Apkarian et al., 2005; Christmann et al., 2007). In contrast, the later positivity, which concurs with the typical P3 period (Yamaguchi and Knight, 1991), has been linked to rather top-down regulated affective and cognitive evaluation processing (Christmann et al., 2007; Kenntner-Mabiala et al., 2008; Valentini et al., 2013). In the present study, amplitudes during both periods were similar for the acquisition trainings of CS set 1, set 2, and set 3, providing evidence that repeated US presentations did not weaken somatosensory processing and attentional engagement with the aversive shock. In sum, peripheral physiology and ERP markers provide evidence that the US induced elevated arousal and increased recruitment of attentional resources during all three acquisition training phases. Importantly, unconditioned responses did not habituate over time.

Rapid learning about changing threat contingencies allows to predict harm in the future and can be critical for survival (LeDoux and Daw, 2018). A crucial goal of attention is to facilitate and accelerate the detection of potential danger (Mogg and Bradley, 1998; Wieser and Keil, 2020). Nevertheless, hypervigilance (Parsons and Ressler, 2013), biased attention toward threat (Burris et al., 2019), delayed attentional disengagement from threat (Amir et al., 2003), and overgeneralization of threat to harmless stimuli (Dunsmoor and Paz, 2015; Nelson et al., 2015b) are core symptoms of several disorders related to clinical fear. Specifically, patients with anxiety disorders display heightened and less flexible neural reactivity to threat (Moser et al., 2008; Mueller et al., 2009; MacNamara and Proudfit, 2014; Kujawa et al., 2015), which may

also be a meaningful predictor for treatment outcome (Stange et al., 2017). Furthermore, faster fear conditioning (Lissek et al., 2005) and delayed fear extinction (Duits et al., 2015) have been discussed as potential mediators in the etiology of anxiety disorders. Although there is evidence for attentional biases in clinical fear, some studies report contradictory findings (Holmes et al., 2008; Mueller et al., 2009; Weinberg and Hajcak, 2011; Weinberg et al., 2016). Moreover, the precise temporal dynamics of attentional threat biases and underlying mechanisms remain largely unknown (MacNamara et al., 2013). In the present study, we introduce sequential-set fear conditioning as a suitable tool to study the speed of neural threat learning. Thus, our novel paradigm may open new avenues to explore which processing stages contribute to aberrant threat processing in pathological fear. This knowledge might, in turn, lay the foundation to design more focused and tailored interventions to efficiently reduce pathological processing biases and to improve attentional control (Cisler and Koster, 2010; Wieser and Keil, 2020).

Although our data provide striking insights into the temporal unfolding of brain circuits during fear learning, there are some limitations. Strengthening the validity of our results, successful fear acquisition could even be probed within individual stimulus sets (e.g., $CS+_1$ versus $CS-_1$). For only the 33–60 ms period, averaging across all CS+/CS- sets was required to detect significantly enhanced CS+ amplitudes. This finding converges with our observation that short-latency effects only occurred during the last five conditioning trials, i.e., the signal-to-noise ratio is insufficient for a single CS set. It should also be kept in mind that there are more trials in the extinction training than in the acquisition training. This imbalance impedes the direct comparison between both experimental stages. Due to the limited spatial resolution of EEG, anatomical correlates of the reported neural processes remain vague, and future studies should combine sequential-set conditioning with simultaneous EEG-fMRI.

5. Conclusion

In conclusion, *sequential-set* fear conditioning provides a powerful design to unravel spatio-temporal dynamics of neural processes during learning about threats. By averaging across CS+/CS- sets, we guarantee a sufficiently high number of trials to detect changes in associative strength during learning and to study habituation-probe neural processes that are of particular relevance for the formation of emotional memories. While several studies have investigated electrocortical correlates of fear conditioning (Miskovic and Keil, 2012), the learning curve of neural processes has so far been neglected in human research. Our paradigm provides a valuable tool to further our understanding of the temporal unfolding of early (< 100 ms), mid-latency, and late neural processes. Developing a more detailed understanding about temporal characteristics of fear learning may have broad implications on neurobiological models of pathological fear and help to identify neurophysiological treatment targets in anxiety and related disorders.

Author contributions

M.F.J.S., A.W., M.M., B.S., and E.M.M. conceived and designed the study paradigm. M.F.J.S., A.W., and M.M. collected and preprocessed the data. M.F.J.S., A.W., M.M., and E.M.M. analyzed the data. M.F.J.S. and E.M.M. interpreted the data. M.F.J.S. and E.M.M. drafted the manuscript, and A.W., M.M., and B.S. provided critical revisions. M.F.J.S. created the figures. M.F.J.S. made the data, analysis scripts, code-books, and research materials publicly available. All of the authors discussed the results, commented on the article, and approved the final manuscript for submission.

Declaration of Competing Interest

The authors declare no competing financial interests.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.neuroimage.2020.117569. Open Practices: De-identified data (for analyses described in this manuscript along with a code-book and the data analysis scripts) and research materials have been made publicly available via Zenodo and can be accessed at https://doi.org/10.5281/zenodo.4294603.

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4.4 Manuscript 4:

Alpha-2 Adrenoreceptor Antagonist Yohimbine Potentiates Consolidation of Conditioned Fear

Sperl, M. F. J., Panitz, C., Skoluda, N., Nater, U. M., Pizzagalli, D. A., Hermann, C., & Mueller, E. M. (submitted). Alpha-2 adrenoreceptor antagonist yohimbine potentiates consolidation of conditioned fear. Submitted to *Neuropsychopharmacology*.

Open Data and **Open Materials** will be available online at *Zenodo* after acceptance.

Scientific Recognition:
I received an **SPR Poster Award** from the **Society for Psychophysiological Research (SPR)** for this project, awarded at the 57th Annual Meeting 2017 at the Hofburg Vienna, Austria.

Abstract

Hyperconsolidation of aversive associations and poor extinction learning have been hypothesized to be crucial in the acquisition of pathological fear. Previous animal and human research has pointed to the potential role of the catecholaminergic system, particularly noradrenaline and dopamine, in acquiring emotional memories. Here, we investigated in a between-subjects design with three groups whether the noradrenergic alpha-2 adrenoreceptor antagonist yohimbine and the dopaminergic D2 receptor antagonist sulpiride modulate long-term fear conditioning and extinction in humans. Fifty-five healthy male students were recruited. The final sample consisted of N = 51 participants who were explicitly aware about the CS–US contingencies after fear acquisition. The participants were then randomly assigned to one of the three groups and received either yohimbine (10 mg, n = 17), sulpiride (200 mg, n = 16), or placebo (n = 18) between fear acquisition and extinction. The yohimbine group showed increased alpha-amylase activity, confirming a successful manipulation of central noradrenergic release. Recall of conditioned (non-extinguished CS+ versus CS-) and extinguished fear (extinguished CS+ versus CS-) was assessed one day later, while a 64-channel electroencephalogram (EEG) was recorded. Elevated fear-conditioned bradycardia and larger differential amplitudes of the N170 and LPP components in the event-related potential indicated that yohimbine treatment (compared with a placebo and sulpiride) enhanced fear recall during day 2. These results suggest that yohimbine potentiates cardiac and central electrophysiological signatures of fear memory consolidation. They thereby elucidate the key role of noradrenaline in strengthening the consolidation of conditioned fear associations, which may be a key mechanism in the etiology of fear-related disorders.

Keywords: fear conditioning; noradrenaline; norepinephrine; dopamine; yohimbine; sulpiride

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Introduction

Heightened attention toward threat facilitates survival, but can also contribute to clinical fear [1]. While fear conditioning is construed as a core learning process in the etiology of anxiety and stressor-related disorders [2], extinction learning is critical for the success of exposure therapy [3]. Noradrenergic (norepinephrine, NE) activation, as induced by emotionally arousing experiences, is crucial for the formation and consolidation of new memory traces [4,5]. Exaggerated noradrenergic stimulation of the amygdala, hippocampus, and prefrontal brain areas plays a pivotal role in pathological fear, presumably mediated through aberrant conditioning and extinction [6,7]. Notably, overconsolidation of memories about life-threatening events due to amplified noradrenergic transmission may lead to intrusive memories [8], which are hard to extinguish [9,10]. Heightened threat responsiveness in PTSD is mediated by hyperactivity of the locus coeruleus [11], the principal site for NE synthesis in the brain [12].

Rodent research has shown that stress-induced NE is critical for the consolidation of emotional memories [7,13]. Optogenetic activation of locus coeruleus fibers leads to enhanced fear conditioning, presumably via NE release into the amygdala [14]. The drug yohimbine acts as an antagonist at α2 autoreceptors in the locus coeruleus and stimulates NE release [15–17]. Of note, yohimbine facilitates fear consolidation [18] and generates a PTSD-like fear memory in rodents [19,20]. In humans, yohimbine strengthens consolidation of fear-conditioned startle responses [21,22], in line with an hyperconsolidation hypothesis in PTSD [8].

In addition to its facilitating effect on fear consolidation, yohimbine may also enhance extinction [23–25]. This could have important clinical implications for the augmentation of exposure therapy [26]. However, the results of rodent studies have been contradictory [27], and there is even evidence that yohimbine may enhance fear relapse [28]. Studies in humans suggest that yohimbine facilitates exposure therapy in PTSD [29], social anxiety disorder [30], and claustrophobia [31]. However, others failed to replicate these effects for patients with a fear of flying [32,33] and acrophobia [33].

As outlined above, there is evidence that yohimbine facilitates fear consolidation. In contrast, some researchers have used yohimbine as a pharmacological complement to augment extinction learning during exposure therapy, but yielded mixed results [27]. Experimental and therapeutic studies have either focused on fear consolidation or aimed at boosting extinction, but the two mechanisms have not been differentiated adequately. Here, we fill this gap by assessing yohimbine effects in an established paradigm [34] that allows us to distinguish the mechanisms specific to fear consolidation and extinction recall.

Furthermore, it remains unclear how yohimbine affects neural threat circuits in humans. Previous studies have tended to concentrate on peripheral measures [21,22,29,35,36]; in the current study, we combined peripheral (skin conductance, heart rate) and central (electroencephalogram, EEG) physiology to measure the effects of yohimbine. We were interested specifically in the N170 component and the late positive potential (LPP). The LPP is a reliable marker of conditioned fear [37–39], and the N170 has also been amplified when faces served as CS [39–41].

Besides its noradrenergic impact, yohimbine acts as antagonist at dopaminergic D2 receptors [27,42,43]. In particular, yohimbine may block D2 autoreceptors and lead to elevated cortical dopamine (DA) levels [27,44,45]. So far, it has not been ascertained whether the effects of yohimbine can be ascribed to noradrenergic or dopaminergic signaling. As with noradrenergic pathways, the dopaminergic system plays a crucial role in acquiring emotional memories [46,47]. To disentangle effects of yohimbine on NE and DA, we applied a between-subjects design with *three* groups. In addition to the yohimbine and placebo groups, a third group received the dopamine D2 receptor antagonist sulpiride. We reasoned that, if yohimbine effects are driven by NE (versus DA) transmission, the pharmacological effects on fear conditioning and extinction should be specific to the yohimbine group, and should not generalize to the sulpiride group.

In sum, animal and initial human studies suggest that yohimbine can boost fear consolidation, but neurophysiological mechanisms have rarely been studied in humans. As has been noted, there is also tentative evidence that yohimbine may facilitate fear extinction, and thus enhance the efficacy of exposure therapy. Our study aims to elucidate (1) how yohimbine *differentially* affects fear consolidation and extinction learning; (2) which *brain correlates* underlie these mechanisms; and (3) whether the effects of yohimbine are driven specifically by *noradrenergic* stimulation.

Materials and Methods

Subjects

We recruited 55 healthy male students who were then randomly assigned to the three above-mentioned groups (exclusion criteria in the *Supplemental Material A*). One participant did not complete the study. Three subjects were excluded as they fulfilled our criterion of "unlikely explicit contingency awareness" (i.e., higher awareness ratings for CS- than CS+ after acquisition, as defined by [48]). Therefore, the final sample consisted of $N = 51$ participants ($n = 17$ yohimbine group, $n = 16$ sulpiride group, $n = 18$ placebo group). We tested males only, because yohimbine's neural effects are sex-dependent [49] and estrogen levels modulate fear/extinction recall [50]. The study protocol was approved by the ethics committee of the German Psychological Society (DGPs).

Experimental Fear Conditioning and Extinction Paradigm

Participants underwent a well-established 2-day fear conditioning/extinction paradigm [34] with acquisition and extinction stages on day 1 and a recall test on day 2 (see Figure 1A). After extinction, participants completed a gambling task [51] unrelated to the current study.

During acquisition, two CS+ (CS+E, CS+N) and two CS- (CS-E, CS-N) were presented 60 times. Neutral faces [52] served as CSs (*Supplemental Material B*). Both CS+ co-terminated with a white noise US [53] at a partial reinforcement rate of 50%.

Three hours after acquisition, subjects began extinction training. One of the two CS+ (i.e., the "extinguished" CS+, CS+E) and one of the two CS- (i.e., the CS-E) were presented 40 times each in random order, to extinguish threat responses to the CS+E. The other two CSs (i.e., the "nonextinguished" CSs, CS+N and CS-N) and the US were not presented during the extinction phase, to leave learned responses to CS+N and CS-N fully intact. A novel face was shown 20 times to maintain some variability of stimuli.

During a recall test approximately 26h after extinction, all stimuli (i.e., CS+E, CS+N, CS-E, CS-N) were presented 60 times each without any US presentation. By computing differential responses for extinguished (CS+/-E) and nonextinguished (CS+/-N) stimuli separately, extinction recall could be distinguished from fear recall on day 2.

Pharmacological Challenge: Yohimbine, Sulpiride, and Placebo

Between acquisition and extinction, participants received in a double-blind manner an oral dose of either yohimbine hydrochloride (10 mg), sulpiride (200 mg), or a placebo (*Supplemental Material C*). Yohimbine (45–75 min [54–58]) and sulpiride (3–4 h [59–61]) vary in the time they take to reach peak plasma concentrations. To ensure peak plasma levels at a similar time prior to extinction, each participant ingested two capsules (Figure 1B). Participants in the sulpiride group received sulpiride 3 h prior to extinction at t_1 and a placebo pill at t_2. Participants in the yohimbine group received yohimbine 45 min prior to extinction at t_2 and a placebo pill at t_1. For participants in the placebo group, both capsules contained placebo pills.

Yohimbine. The indole alkaloid yohimbine promotes central and peripheral NE release [62]. In the brain, yohimbine acts as antagonist at presynaptic α2 adrenoceptors in the locus coeruleus [15]. Blocking these inhibitory autoreceptors leads to increased locus coeruleus firing and NE release

[16,17]. Beyond the NE system, yohimbine also acts on dopaminergic D2 receptors [27,42]. To confirm its successful influence on central NE [63–65], we assessed salivary α-amylase activity (sAA, *Supplemental Material D*).

Sulpiride. The substituted benzamide sulpiride acts as a selective antagonist at pre- and postsynaptic dopaminergic D2 receptors [59]. Sulpiride does not appear to significantly block other receptor types, such as NE receptors [66,67]. The effects of sulpiride on DA depend partly on the dose chosen [68–70]. High doses (> 400mg) are thought to exert effects primarily on postsynaptic D2 receptors [71,72], thus reducing dopaminergic action [73]. In contrast, low doses of sulpiride (e.g., 100–200mg) appear to block mainly presynaptic autoreceptors, which is assumed to result in a net *stimulatory* effect on dopaminergic transmission [74,75]. Here, we used a single acute dose of 200 mg [76–78] to *increase* DA [75,79].

Affective CS Ratings

Participants were asked to rate each CS with regard to its associated arousal and valence (see *Supplemental Material E/G*). We expected higher ratings of arousal and negative valence after fear acquisition for both CS+ (CS+E, CS+N) compared with both CS- (CS-E, CS-N), which was assessed by *Contingency* (CS+, CS-) x *Later Extinction Status* (E, N) x *Group* (yohimbine, sulpiride, placebo) ANOVAs. For extinction, we computed a *Contingency* (CS+E, CS-E) x *Time* (before, after extinction) x *Group* ANOVA, as we expected a decrease of conditioned responses. At the beginning of the day 2 recall, a *Contingency* x *Extinction Status* x *Group* ANOVA was carried out. We expected larger conditioned responses for nonextinguished (CS+N versus CS-N) compared with extinguished stimuli (CS+E versus CS-E).

Physiological Data

Peripheral physiological data (skin conductance and electrocardiogram) were collected during all stages. Participants received either yohimbine, sulpiride, or a placebo between acquisition and extinction. We were interested specifically in the pharmacological influences on neural threat signatures during subsequent extinction and fear/extinction recall 26h later. Hence, in addition to peripheral measures, we recorded EEG during the day 1 extinction and day 2 recall stages (*Supplemental Material F/G*).

Peripheral Physiology. Successful fear conditioning should be accompanied by higher SCRs [34] and relative cardiac deceleration ("fear-conditioned bradycardia" [38]) for both CS+ (CS+E, CS+N) compared with both CS- (CS-E, CS-N). For acquisition, we computed *Contingency* (CS+, CS-) x *Later Extinction Status* (E, N) x *Group* ANOVAs. For extinction, we calculated *Contingency* x *Time* x *Group* ANOVAs to assess whether conditioned responses (CS+E versus CS-E) declined from early (first ten) to late (last ten) trials [80,81]. Successful fear and extinction recall on day 2 would be evident from larger physiological responses for CS+N compared with CS-N, while responses following CS+E and CS-E should be similar. We ran a *Contingency* x *Extinction Status* x *Group* ANOVA for the day 2 data.

Electroencephalography. The EEG was recorded from 64 channels. We quantified the N170 (145–185ms at left/right occipito-temporal channels T7/8, TP7/8, TP9/10, P7/8, PO9/10) and LPP (400–800ms at parieto-occipital channels P1, Pz, P2, PO3, POz, PO4, O1, Oz, O2) of the event-related EEG (*Supplemental Material F*). Extinction ANOVAs included the factors *Contingency* x *Hemisphere* (left, right; only for N170 analyses) x *Electrode* x *Group*. The ANOVAs for day 2 fear/extinction recall contained the additional factor *Extinction Status*.

Data and Code Availability

De-identified data along with a code-book and analysis scripts are posted at [*url to the Zenodo repository will be provided after acceptance of this manuscript*].

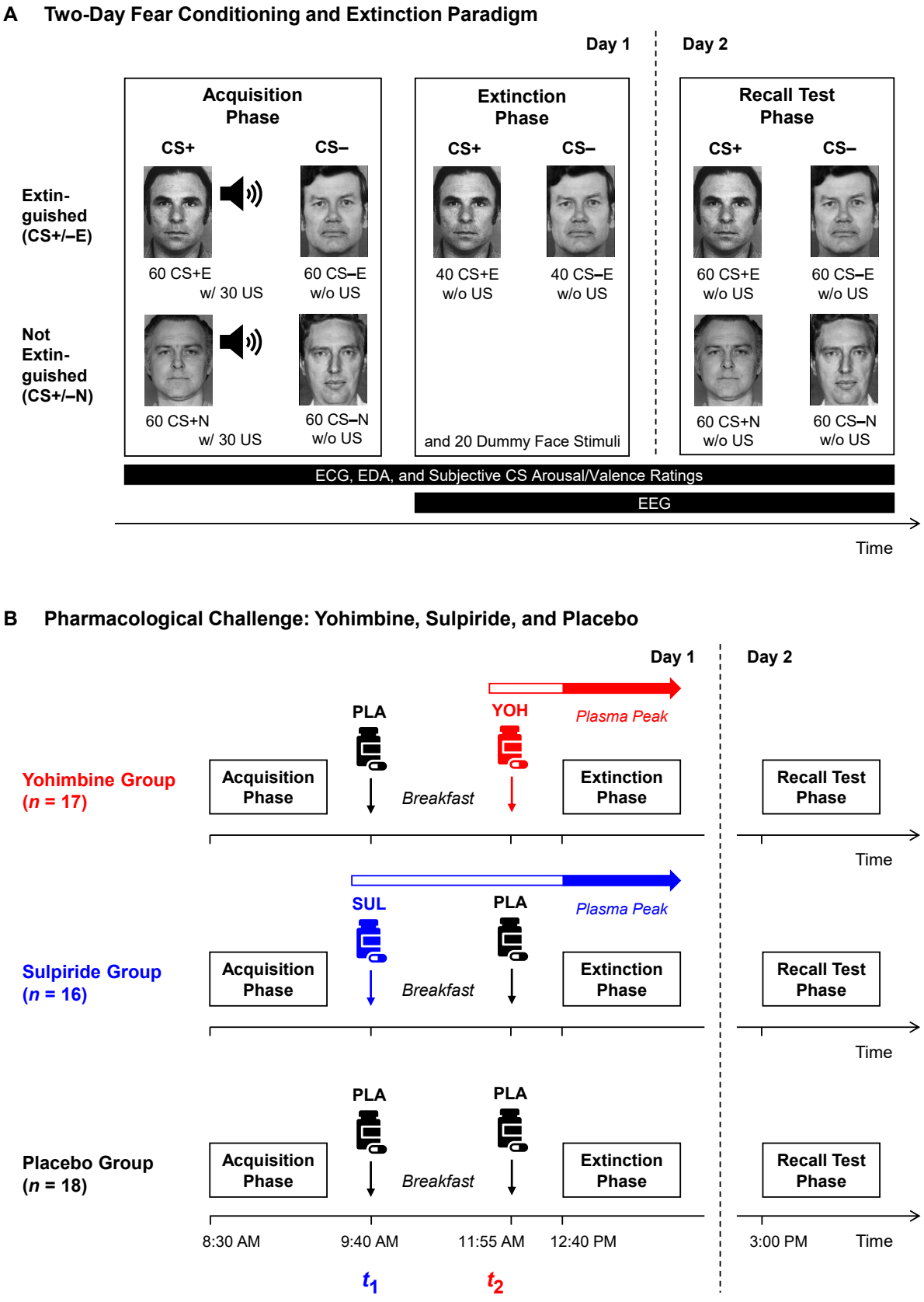

Figure 1. Experimental fear conditioning and extinction paradigm used in the present study. (A) Stimulus types and number of presentations during the three experimental phases. During acquisition training on the first day, two conditioned stimuli (two CS+: CS+E and CS+N) were reinforced with ("w/") an aversive unconditioned stimulus (US), which consisted of an unpleasant white noise burst (contingency of 50%).

Conversely, two other CS- (CS-E and CS-N) were not paired with the US ("w/o"). Afterward, participants underwent extinction training, during which only one CS+ ("extinguished" CS+, CS+E) and one CS- (CS-E) were shown. The CS+N and CS-N ("nonextinguished" CS+/-) were not presented during extinction training. A novel face ("Dummy Stimulus") was shown to maintain some variability of stimuli. On the second day, all stimuli were presented during a recall test without US presentation. To identify effects specific to fear versus extinction recall, we compared differential responses for nonextinguished (CS+N minus CS−N) stimuli with differential responses for extinguished stimuli (CS+E minus CS−E). Electrocardiogram (ECG) and electrodermal activity (EDA) were assessed during all stages. In addition to these peripheral measures, we recorded EEG during the day 1 extinction and day 2 recall stages. (B) Pharmacological challenge. Between fear acquisition and extinction stages, participants received an oral dose of either 10 mg of yohimbine HCl (YOH, $n = 17$), 200 mg of sulpiride (SUL, $n = 16$), or a placebo pill (PLA, $n = 18$). All participants were tested at the same time of day to control for effects of circadian rhythms. Note that both substances (yohimbine and sulpiride) differ in the time they take to reach peak plasma concentration. Thus, sulpiride was administered at 9:40 AM (= t_1), and yohimbine at 11:55 AM (= t_2), to ensure that participants from both experimental groups reached peak plasma levels at a similar point. To guarantee successful blinding for experimenters and participants, each participant received two capsules (e.g., participants in the sulpiride group received the active substance sulpiride at t_1 and a placebo pill at t_2; participants in the placebo group received two placebo pills). All participants received a standardized light breakfast (water and 1–2 bread rolls with jam, hazelnut cocoa spread, cheese, or sausage) between the two capsules.

Results

Manipulation Check Drug Administration: Salivary α-Amylase

Yohimbine administration (versus placebo) increased sAA activity (Figure 2) directly before ($t(32) = 2.34$, $p = .026$) and after extinction ($t(32) = 2.26$, $p = .032$), confirming the successful manipulation of NE release. There was no difference between groups before ingestion of the first capsule ($p = .820$) and before day 2 recall ($p = .871$).

Day 1 Fear Acquisition

Affective CS ratings and peripheral physiological responses confirmed successful fear conditioning. The two CS+ (CS+E and CS+N), relative to the two CS- (CS-E and CS-N), evoked larger SCR amplitudes (*Contingency* main effect, $F(1,48) = 15.87$, $p < .001$) and stronger cardiac deceleration ("fear-conditioned bradycardia"; $F(1,47) = 44.94$, $p < .001$), and were assessed as significantly more arousing ($F(1,48) = 23.46$, $p < .001$) and unpleasant ($F(1,48) = 27.36$, $p < .001$). There were no significant interactions including the factors *Later Extinction Status* or *Group* (all ps $\geq .318$).

Day 1 Fear Extinction

The *Contingency* x *Time* x *Group* ANOVAs on CS arousal ratings and CS-evoked SCRs revealed significant *Contingency* main effects. Specifically, the CS+E was still rated as significantly more arousing than CS-E ($F(1,48) = 20,89$, $p < .001$), and generated elevated SCRs ($F(1,48) = 4.09$, $p = .049$). ANOVAs on valence ratings, heart period, and N170/LPP components did not yield significant effects involving *Contingency* (ps $\geq .081$).

During extinction, we did not observe significant interactions with the *Group* factor (ps $\geq .081$). This finding is in keeping with previous studies suggesting that yohimbine affects mainly *consolidation* processes [21,22], which occur predominantly during sleep [82]; therefore, yohimbine effects would be expected especially on day 2.

Day 2 Recall: Affective Ratings and Peripheral Physiological Data

The *Contingency* x *Extinction Status* x *Group* ANOVA for arousal ratings at the beginning of day 2 fear/extinction recall showed a significant *Contingency* main effect ($F(1,48) = 25.742$, $p <$

.001). Both CS+E and CS+N were rated as significantly more arousing compared with CS-E and CS-N. Likewise, we observed elevated SCRs for both CS+ compared with both CS- (*Contingency* main effect, $F(1,48) = 8.79$, $p = .005$). The ANOVA on valence ratings did not yield any significant effects ($ps \geq .159$). Contrary to our hypotheses, there were no significant interactions with the *Extinction Status* or *Group* factors ($ps \geq .215$) for affective ratings and SCRs.

The ANOVA on heart period data (Figure 3), however, revealed a significant *Contingency* x *Extinction Status* x *Group* interaction ($F(2,48) = 4.27$, $p = .020$, $\eta_p^2 = .151$). To assess further the influence of the pharmacological manipulation on fear/extinction recall, we ran separate follow-up *Contingency* x *Extinction Status* ANOVAs for each of the three groups. In contrast to prior studies [38,83], we observed no significant main effects or interactions within the placebo ($ps \geq .261$) and sulpiride ($ps \geq .370$) groups; this indicates an absence of fear recall. Importantly, only the yohimbine group showed a significant *Contingency* x *Extinction Status* interaction ($F(1,16) = 4.70$, $p = .046$, $\eta_p^2 = .227$). For the yohimbine group, differential fear responses were significantly greater for nonextinguished versus extinguished stimuli. In particular, the nonextinguished CS+N was associated with stronger cardiac deceleration than the CS-N ($t(16) = 2.68$, $p = .016$), reflecting successful fear recall. Conversely, there was no difference in the cardiac deceleration response between the extinguished CS+E and CS-E ($t(16) = –0.17$, $p = .870$). In conclusion, yohimbine administration on day 1 was associated with enhanced recall of fear-conditioned bradycardia on day 2.

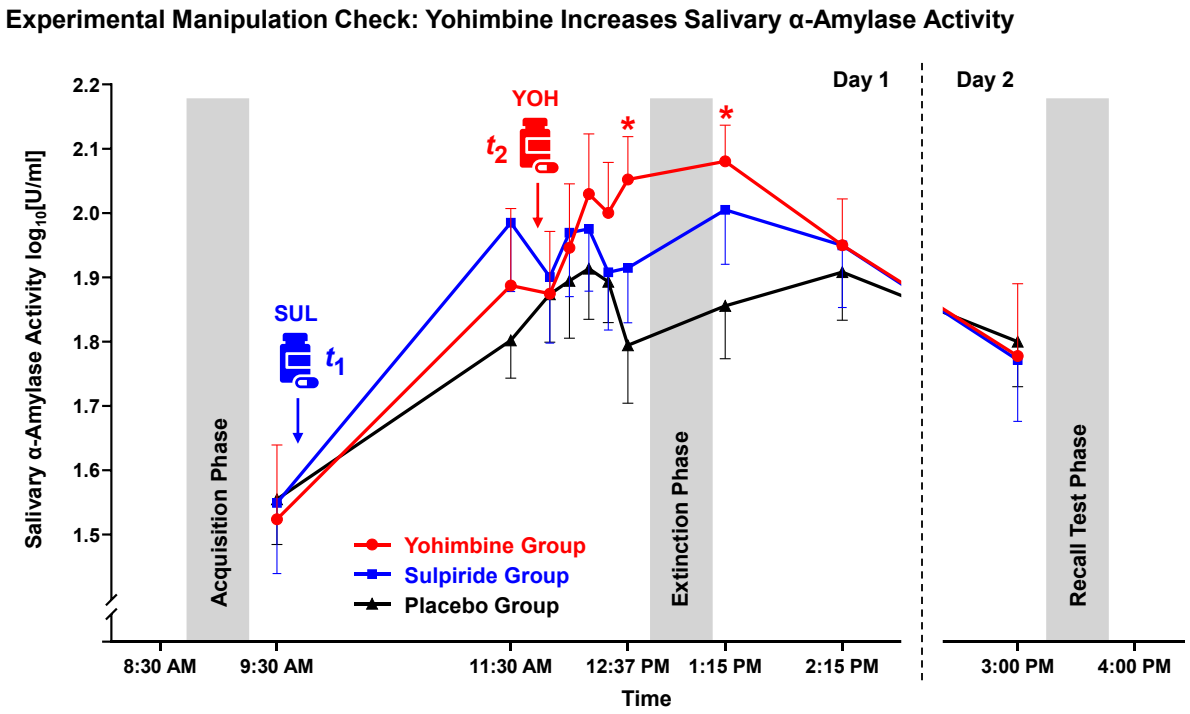

Figure 2. Between fear acquisition and extinction stages, participants received an oral dose of either 200 mg of sulpiride (at t_1; $n = 16$), 10 mg of yohimbine HCl (at t_2; $n = 17$), or a placebo pill ($n = 18$). Salivary α-amylase activity (sAA) was assessed to confirm the successful influence of yohimbine on central noradrenaline (NE) release. Saliva samples were collected by using the passive drool method on both days at several time points (day 1: 9:30 AM, 11:30 AM, 11:57 AM, 12:07 PM, 12:17 PM, 12:27 PM, 12:37 PM, 1:15 PM, 2:15 PM; day 2: 3:00 PM). Compared with the placebo, yohimbine administration was associated with significantly elevated sAA activity directly before (12:37 PM) and after (1:15 PM) extinction training. All participants were tested at the same time of day to control for effects of circadian rhythms. *$p \leq .05$.

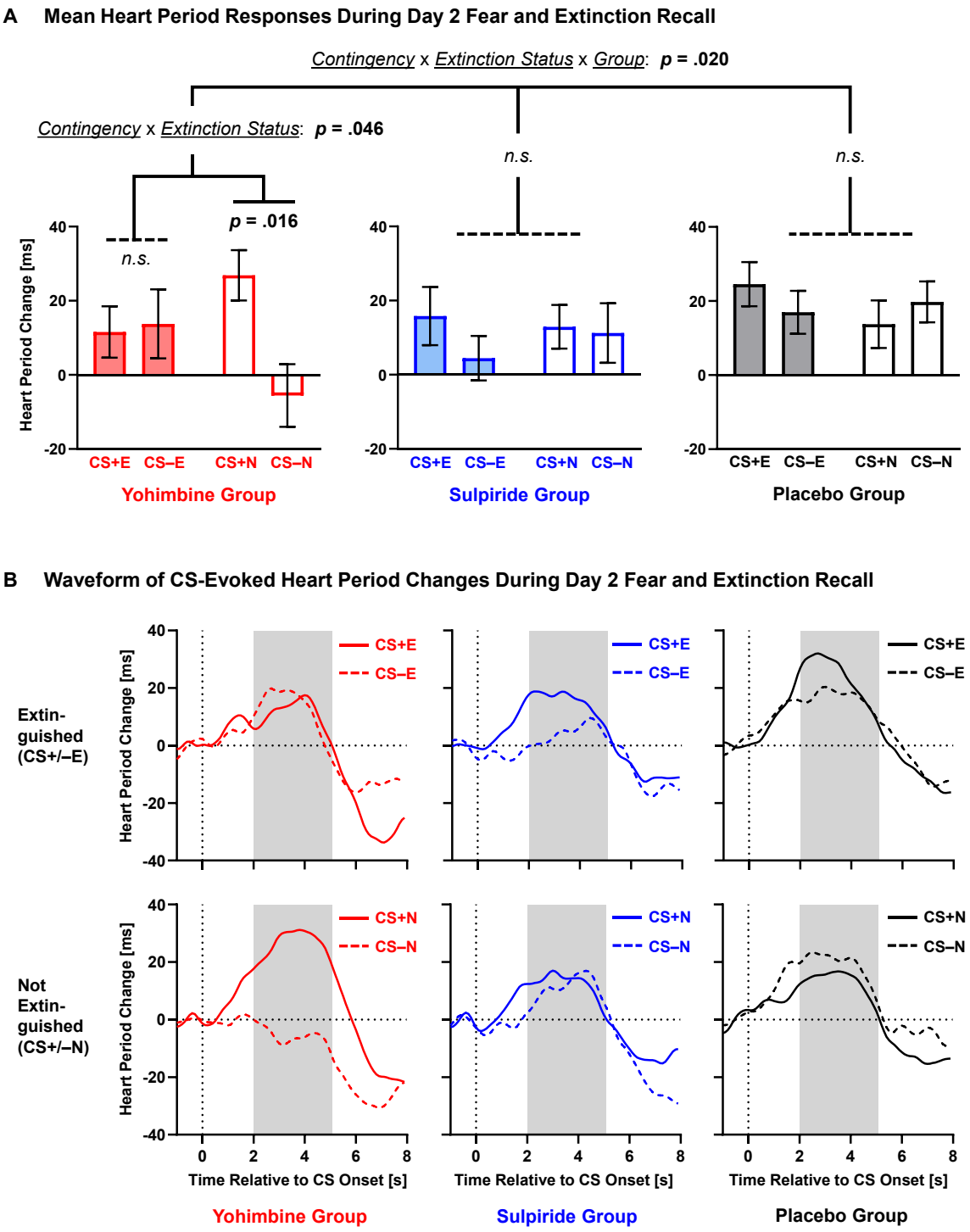

Figure 3. Fear-conditioned bradycardia (mean heart period change 2–5 s post-CS) during day 2 recall. (A) The ANOVA for CS-evoked heart period changes revealed a significant *Contingency* x *Extinction Status* x *Group* interaction. Only the yohimbine group showed stronger cardiac deceleration for the nonextinguished CS+N compared with CS-N, indicating enhanced recall of fear-conditioned bradycardia. Mean (± within-subjects *SEM*, adjusted within each group [142]) heart period changes after CS onset are displayed. (B) The waveform of CS-evoked heart period changes is shown for extinguished (CS+E, CS-E; upper panels) and nonextinguished (CS+N; CS-N; lower panels) stimuli, separately for the yohimbine (n = 17; left panels), sulpiride (n = 16; middle panels), and placebo groups (n = 18; right panels). The time series of the interbeat interval was segmented into epochs ranging from –1 to 8 s relative to the CS onset, baseline-corrected (1s pre-CS), and averaged across trials for each CS type. Gray-shaded areas indicate time windows for statistical analyses.

Day 2 Recall: Electroencephalographic Data

N170. EEG responses closely mirrored the influence of yohimbine on fear-conditioned bradycardia. The ANOVA on N170 amplitudes (Figure 4) revealed a significant *Contingency* x *Extinction Status* x *Hemisphere* x *Electrode* x *Group* interaction ($F(8,192) = 2.60$, $p = .016$, $\eta_p^2 = .098$). Unexpectedly (but in line with our heart period data), follow-up *Contingency* x *Extinction Status* x *Hemisphere* x *Electrode* ANOVAs for the placebo and sulpiride groups did not reach significance (with the exception of *Electrode* main effects, $ps \leq .001$). However, in the yohimbine group, we observed a significant *Contingency* x *Extinction Status* x *Hemisphere* x *Electrode* interaction ($F(4,64) = 5.30$, $p = .001$, $\eta_p^2 = .249$). Convergent with prior observations that N170 responses are usually more pronounced in the right brain hemisphere [84,85], significant *Contingency* x *Extinction Status* interactions were confirmed at three *right* hemispheric electrodes: TP10 ($p = .013$), P8 ($p = .006$), and PO10 ($p = .040$). The N170 amplitude was significantly larger (more negative) for the nonextinguished CS+N compared with CS-N (TP10: $p = .033$; P8: $p = .008$¸ PO10: $p = .020$). In contrast, there was no difference between the extinguished CS+E and CS-E (TP10: $p = .517$; P8: $p = .496$¸ PO10: $p = .774$).

LPP. For the LPP period (Figure 5), the ANOVA showed a significant *Contingency* x *Extinction Status* x *Group* interaction ($F(2,48) = 3.43$, $p = .041$, $\eta_p^2 = .125$). Follow-up ANOVAs for the placebo and sulpiride groups indicated significant *Electrode* main effects ($ps \leq .024$), but no further main effects or interactions ($ps \geq .198$). Only the LPP ANOVA for the yohimbine group revealed a significant *Contingency* x *Extinction Status* interaction ($F(1,16) = 4.61$, $p = .047$, $\eta_p^2 = .224$); this complemented our N170 results. We observed larger LPP amplitudes for CS+N compared with CS-N ($t(16) = 3.15$, $p = .006$) within the yohimbine group. Conversely, there was no significant difference between LPP responses following CS+E and CS-E ($t(16) = 1.25$, $p = .229$).

Discussion

Noradrenergic hyperactivity plays a pivotal role in fear-related disorders [4,86,87]. Our primary goal was to elucidate NE effects on brain correlates of fear and extinction consolidation. Between conditioning and extinction, participants received either the α2 adrenoreceptor antagonist yohimbine (which leads to increased noradrenergic stimulation), the D2 receptor antagonist sulpiride (at low dose, which is thought to increase dopaminergic transmission), or a placebo. Sulpiride was added to exclude the possibility that yohimbine effects might be driven by DA, as yohimbine (besides causing marked NE actions) also shows considerable affinity at D2 receptors [42,43]. The next day, we assessed peripheral and neural responses associated with fear and extinction recall. Notably, post-conditioning noradrenergic – but not dopaminergic – stimulation facilitated fear recall one day later, as manifested by fear-conditioned bradycardia and larger N170 and LPP amplitudes.

During day 2 recall, we compared differential responses to nonextinguished (CS+N minus CS-N) with extinguished (CS+E minus CS-E) stimuli to identify effects specific to fear versus extinction recall. Importantly, only participants who received yohimbine showed relative cardiac deceleration (bradycardia) for stimuli that had been fear-conditioned and not extinguished (CS+N compared with CS-N). No effects for this contrast emerged for the placebo and sulpiride groups. Responses after extinguished CS+E were similar to CS-E in each of the three groups. Together, these results indicate that yohimbine selectively strengthened fear consolidation, resulting in robust fear recall on the second day.

Remarkably, neural responses during day 2 closely resembled the effects we observed on fear-conditioned bradycardia. Only participants in the yohimbine group showed significantly larger (more negative) amplitudes of the face-sensitive N170 component for the nonextinguished CS+N compared with CS-N, reflecting fear recall. This effect was absent in the sulpiride and placebo

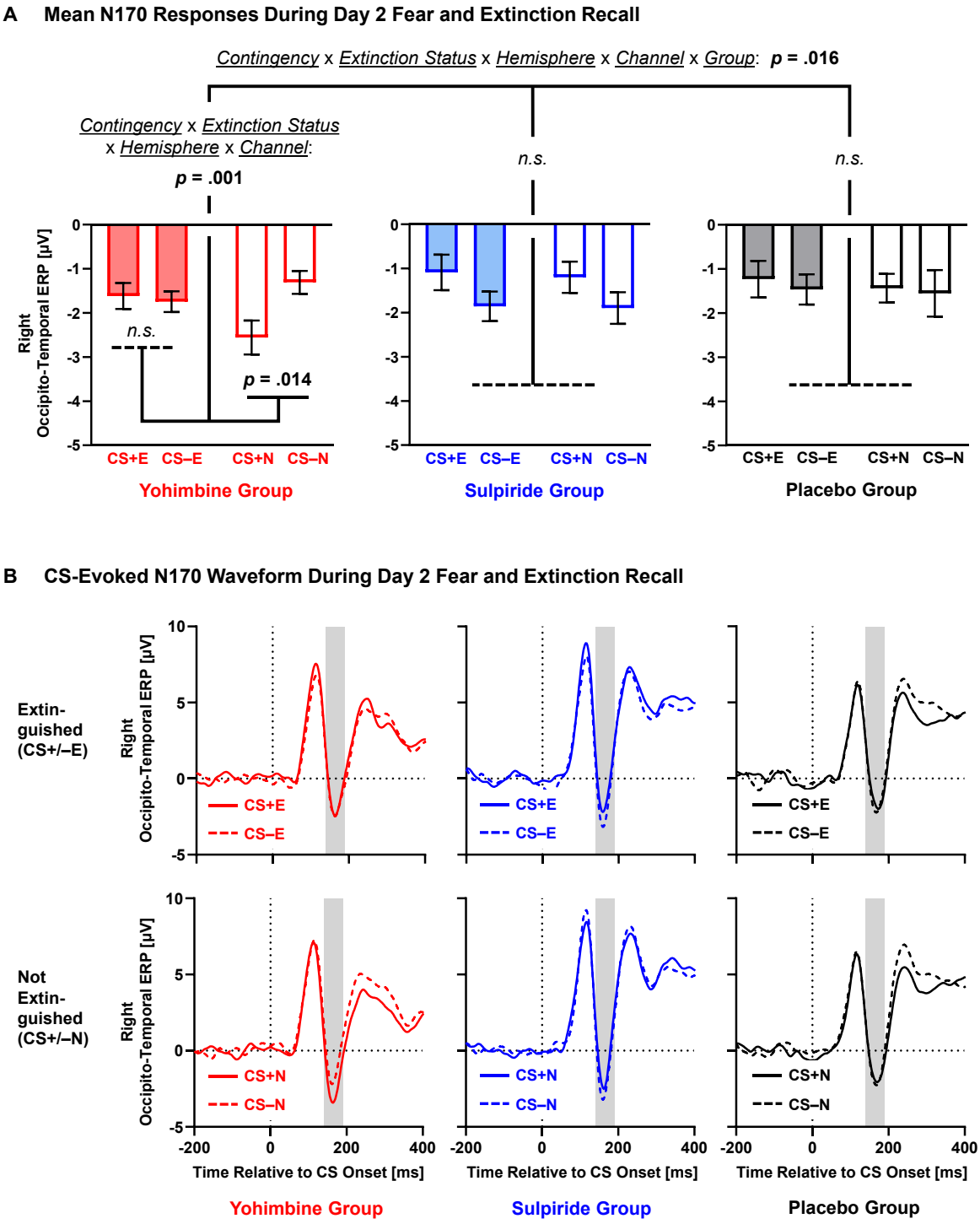

Figure 4. CS-evoked N170 component during day 2 recall. The ANOVA on mean amplitudes (145–185 ms post-CS) yielded a significant *Contingency* x *Extinction Status* x *Hemisphere* x *Electrode* x *Group* interaction. Only the yohimbine group showed significantly larger (i.e., more negative) N170 amplitudes for the nonextinguished CS+N compared with CS-N, and effects were restricted to the channels TP10, P8, and P010 over the right hemisphere. To illustrate (A) mean voltage changes (± within-subjects *SEM*, adjusted within each group [142]) and (B) ERP waveforms, the electrode sites TP10, P8, and P010 were averaged. The EEG data were referenced against Cz, as this central reference highlights better the N170 at occipito-temporal channels [143]. Gray-shaded areas indicate time windows for statistical analyses. The CS-evoked N170 waveform is shown for extinguished (CS+E, CS-E; upper panels) and nonextinguished (CS+N; CS-N; lower panels) stimuli, separately for the yohimbine (n = 17; left panels), sulpiride (n = 16; middle panels), and placebo groups (n = 18; right panels).

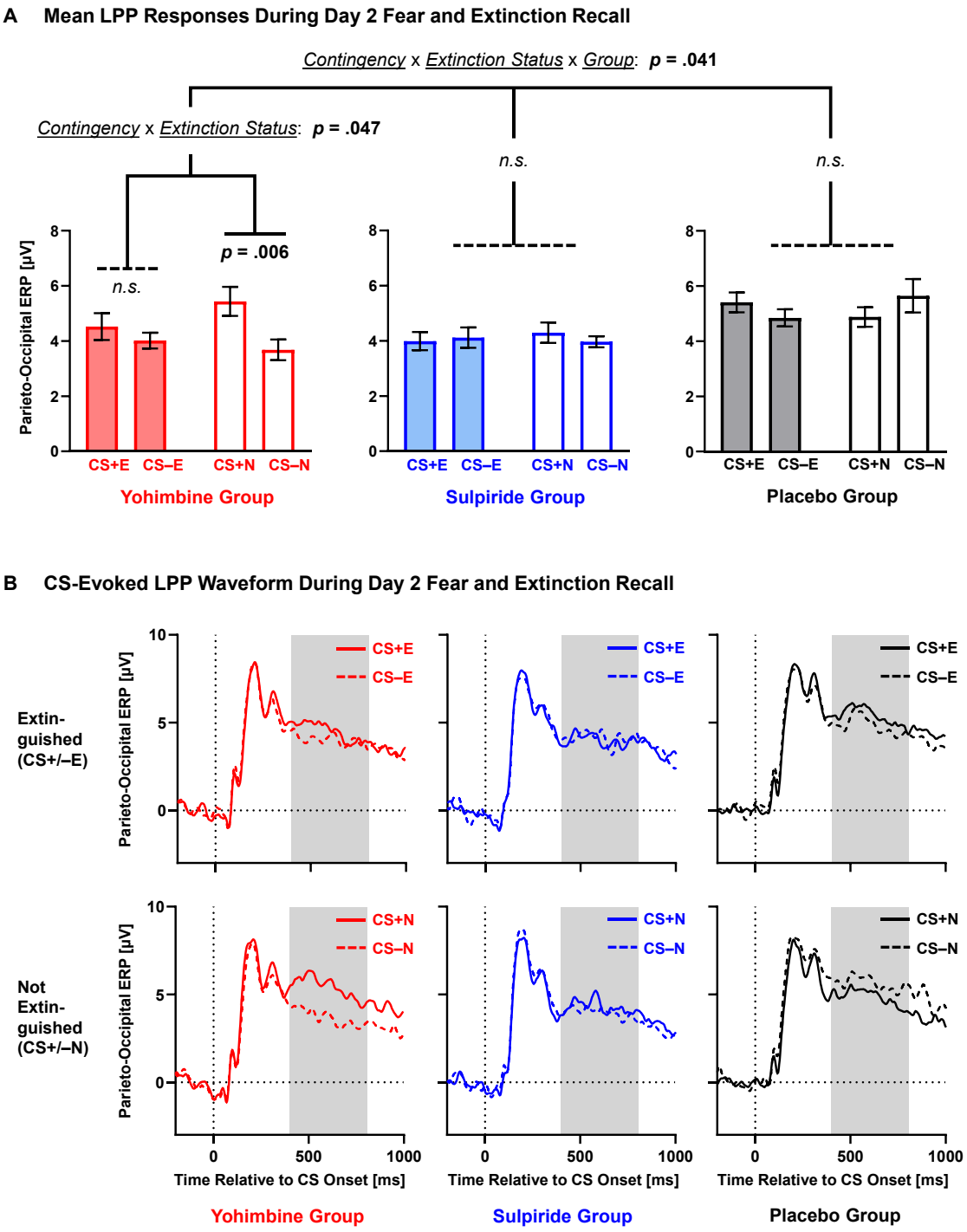

Figure 5. CS-evoked LPP component during day 2 recall. The ANOVA on mean amplitudes (400–800 ms post-CS) yielded a significant *Contingency* x *Extinction Status* x *Group* interaction. Only the yohimbine group showed significantly larger (i.e., more positive) LPP amplitudes for the nonextinguished CS+N compared with CS-N. As there was no significant interaction with the *Electrode* factor, all parieto-occipital channels (P1, Pz, P2, PO3, POz, PO4, O1, Oz, and O2) were averaged to illustrate (A) mean voltage changes (± within-subjects *SEM*, adjusted within each group [142]) and (B) ERP waveforms. The EEG was referenced to the average of TP9 and TP10 (mastoids), which is consistent with the majority of LPP studies [97,100]. The mastoid reference allows emotion-related LPP modulations to be better highlighted [97]. Gray-shaded areas indicate time windows for statistical analyses. The CS-evoked LPP waveform is shown for extinguished (CS+E, CS-E; upper panels) and nonextinguished (CS+N; CS-N; lower panels) stimuli, separately for the yohimbine (n = 17; left panels), sulpiride (n = 16; middle panels), and placebo groups (n = 18; right panels).

groups. The N170 component is a mid-latency, negative-going ERP maximal over occipito-temporal scalp regions, which is particularly large in response to fear-conditioned [39–41,88–91] faces [84,85,92]. Under the assumption that the N170 component is sensitive to variations in attention allocation [93,94], elevated fear recall in the yohimbine group may thus indicate enhanced recruitment of attentional resources to faces that have been fear-conditioned, consolidated under high levels of noradrenergic arousal, and not extinguished on the previous day. Interestingly, we observed larger N170 amplitudes for CS+N versus CS-N only at sensors over the right hemisphere, converging with the lateralization effects reported in previous fear conditioning studies [41,88; but see 40]. N170 amplitudes are typically larger over the right hemisphere [84,85]. This accords with the hypothesis of a right hemispheric advantage in face [95] and danger-related emotion processing [96].

Similar to N170 effects, LPP amplitudes were enhanced for the nonextinguished CS+N versus CS-N, specifically in the yohimbine group. There was no significant difference between CS+N and CS-N in the sulpiride and placebo groups. The LPP is a late-latency parieto-occipital positivity [97,98], indicating sustained attention and elaborated neural processing [99] due to stimulus significance [100]. It is reliably elevated in response to fear-conditioned stimuli [37–39,83,101–105], and is even sensitive to NE-related genetic influences on fear conditioning [83,106]. LPP activity appears to be generated through the locus coeruleus NE system, which potentiates responding to arousing and motivationally significant stimuli [100,107,108]. Collectively, our findings suggest that the administration of yohimbine strengthens neural signatures of conditioned fear that are linked to motivational NE circuits in the brain.

In contrast to some studies reporting threat responses with regard to N170/LPP [37,39,40], we did not find N170/LPP threat responses on day 2 in the placebo group. However, this observation is in line with previous EEG studies that have applied very similar 2-day conditioning paradigms. In two prior datasets [38,109], for example, we were unable to detect reliable conditioning effects on N170 or LPP amplitudes on the second day. In another study [83], LPP amplitudes on day 2 were elevated for CS+N compared with CS-N, but only in individuals of the Val/Val genotype of the *COMT* Val158Met polymorphism. Taken together, these findings suggest that electrocortical threat correlates can only be observed on day 2 after sufficient fear consolidation (e.g., as induced through NE release).

Regarding extinction recall, heart period, N170, and LPP responses did not differ between the extinguished CS+E and CS-E in any of the three groups. The lack of yohimbine effects on extinction learning adds to the considerable heterogeneity of findings from animal [27,28] and human [29–33] studies. While there is converging evidence that NE strengthens fear consolidation, it has been discussed that NE may have bidirectional (i.e., facilitating and inhibiting) effects on extinction [46,87,110]. Nevertheless, we may speculate as to why we did not observe yohimbine effects on extinction. Specifically, animal research suggests that yohimbine leads to *faster* fear extinction, i.e., less trials are needed for successful fear reduction [24]. We used a relatively high number of extinction trials to ensure a sufficient signal-to-noise ratio for the ERP computation [111]. This may have resulted in a ceiling effect, so there may have been little left to be augmented by yohimbine [33]. Furthermore, in contrast with typical animal paradigms [27], acquisition and extinction took place on the same day. A longer interval between both experimental stages might be required to allow for sufficient fear memory consolidation before extinction [112,113].

As discussed earlier, the pharmacology of yohimbine includes noradrenergic, but also dopaminergic effects [27,42,43]. After yohimbine intake, sAA activity increased and was significantly larger relative to the placebo group, reflecting elevated release of central NE [63–65]. To disentangle putative NE and DA effects of yohimbine, another group received the dopamine D2 receptor antagonist sulpiride. Notably, we did not observe any sulpiride effects on fear/extinction recall. Sulpiride has been reported to *facilitate* extinction learning in mice [114], but another study has found *attenuated* fear extinction after sulpiride injection into the rat

amygdala [115]. These divergent findings [114–119] may be explained by a recent study in rats, suggesting that sulpiride can reduce fear *expression*, but has no effect on acquisition/extinction learning [120]. Importantly, the absence of sulpiride effects, together with elevated sAA activity for the yohimbine group, suggests that yohimbine facilitated fear consolidation through heightened NE release.

Hypervigilance is a core symptom of PTSD and other fear-related disorders [106]. It is characterized by abnormally elevated arousal and hyperactivity of the noradrenergic system [121]. Yohimbine experimentally mimics the effects of noradrenergic arousal [4,49]. The NE system is highly vulnerable to sustained and uncontrollable stress, resulting in sensitization and persistent hyperarousal [86,122]. These processes lead to enhanced consolidation of emotional memories, which are more robust, detailed, vivid, and longer-lasting [13,123,124]. Classical conditioning is an etiological mechanism, but not everybody who experiences traumatic events develops a mental disorder [125–127]. Notably, it has been suggested that high arousal levels after traumas play a key role in potentiated consolidation of CS–US associations, ultimately contributing to the development of pathological fear [106,122]. Specifically, higher heart rate shortly *after* a traumatic event has been reported in subjects who subsequently developed PTSD [128,129], which is consistent with overconsolidated memory networks due to heightened arousal [106]. Our data support this hypothesis; they demonstrate that noradrenergic hyperactivity after conditioning boosts fear consolidation. Translating this knowledge into clinical practice, this model would suggest that keeping arousal levels low in the aftermath of traumatic events might be a promising way to prevent later transition to PTSD or other fear-related disorders [122,130]. While our study proposes a notable model to stimulate innovative interventions for reducing pathological hyperconsolidation [131,132], clinical studies are needed to evaluate their efficacy.

The present study has some limitations. To control for potential influences of gonadal hormone fluctuations on NE [133] and fear conditioning [50], female participants were excluded. However, it is important to keep in mind that women are at twofold risk of developing PTSD and other fear-related disorders [134,135]; sex differences in the locus coeruleus NE system may explain elevated arousal levels in females [133]. Further research is needed to clarify whether gonadal hormones modulate our findings.

In addition, EEG has limited spatial resolution. Its excellent temporal accuracy allowed us to capture yohimbine effects on brief neurophysiological processes during N170 and LPP periods, but little is known about brain circuits mediating noradrenergic actions in humans [87]. In rats, NE injection into the amygdala immediately after fear conditioning causes PTSD-like memory [136]. Projections from the locus coeruleus might release NE into the amygdala [46], or (vice versa) rapid amygdala processing may initiate locus coeruleus responses [137]. Although amygdala responses might explain threat-evoked potentiation of the N170 [41] and LPP [138], electrophysiological methods have difficulties isolating neural signals from deep structures [139–141]. Future studies should combine our approach with fMRI to clarify the localization of underlying brain processes.

In conclusion, NE facilitates fear memory consolidation, as quantified with cardiac deceleration and brain responses during the N170 and LPP time windows. Our results offer important neural evidence for yohimbine's noradrenergic effects on fear consolidation in humans. Yohimbine provides a striking laboratory model to elucidate neural mechanisms in the etiology of clinical fear, which may open up promising paths for treatment.

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Author Contributions

E.M.M. conceived the study design and acquired funding. M.F.J.S. and C.P. acquired the data. C.P. programmed the experiment and coordinated the data collection. M.F.J.S., C.P., N.S., and E.M.M. preprocessed and analyzed the data. N.S. and U.M.N conducted biochemical sAA analyses. M.F.J.S. and E.M.M. drafted the manuscript, and C.P., N.S., U.M.N., D.A.P., and C.H. made critical revisions. M.F.J.S. created the figures. M.F.J.S. made the data, analysis scripts, and code-books publicly available. All of the authors interpreted and discussed the results, commented on the article, and approved the final manuscript for submission.

4.5 Manuscript 5:
Fearfulness, Neuroticism/Anxiety, and COMT Val158Met in Long-Term Fear Conditioning and Extinction

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Fearfulness, neuroticism/anxiety, and *COMT* Val158Met in long-term fear conditioning and extinction

Christian Panitz[a,*], Matthias F.J. Sperl[a], Juergen Hennig[b], Tim Klucken[c], Christiane Hermann[b], Erik M. Mueller[a]

[a] University of Marburg, Department of Psychology, Gutenbergstr. 18, 35032 Marburg, Germany
[b] University of Giessen, Department of Psychology, Otto-Behaghel-Str. 10F, 35394 Giessen, Germany
[c] University of Siegen, Department of Psychology, Adolf-Reichwein-Str. 2a, 57076 Siegen, Germany

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ABSTRACT

Individual differences in long-term stability of fear memories are of potential relevance for stable dispositions related to threat processing, such as neuroticism/anxiety and fearfulness. As previous research suggests a prominent role of dopamine for the retention of conditioned and extinguished fear, dopaminergic gene polymorphisms may also relate to individual differences in fear stability. While the *COMT* Val158Met polymorphism causes individual differences in prefrontal dopamine, its associations with human long-term fear extinction are currently unknown. Here, $n = 30/29/28$ healthy male Val/Val, Val/Met and Met/Met carriers, respectively, underwent a two-day differential conditioning paradigm with fear acquisition and extinction on Day 1 and a recall test on Day 2 with recordings of EEG and ECG. Fearfulness but not neuroticism/anxiety predicted fear bradycardia (i.e., heart period slowing) during Day 1 fear acquisition while it did not affect extinction or Day 2 fear recall. In contrast, *COMT* Val158Met significantly modulated Day 2 fear recall as evident in fear bradycardia and Late Positive Potential (LPP) amplitudes while it did not affect Day 1 fear or extinction learning. Furthermore, exploratory analyses revealed that individual differences in fear bradycardia during Day 2 extinction recall depended on Day 1 extinction success. Importantly, this contingency was (a) modulated by *COMT* Val158Met and (b) significantly reduced in high vs. low neuroticism/anxiety. The present study indicates that (a) individual differences in dopaminergic genotypes may affect the long-term stability of fear memories and (b) fearfulness vs. neuroticism/anxiety might play distinct roles in initial fear reactions vs. long-term stability of fear memories, respectively.

1. Introduction

Individual differences in fear learning and reduction are believed to contribute to the development and persistence of dispositional fear, anxiety/neuroticism and anxiety disorders (Duits et al., 2015; Lissek et al., 2005; Milad & Quirk, 2012). In experimental studies, fear learning and reduction are commonly studied with differential fear conditioning and subsequent extinction. Briefly, if a conditioned stimulus (CS) is repeatedly followed by an aversive unconditioned stimulus (US), the CS becomes a conditioned threat cue (CS+) which elicits a conditioned threat response (CR), whereas CSs associated with US absence (CS−) may become safety cues that elicit a relatively reduced CR. Differential responses to CS+ vs. CS− indicate the level of conditioned fear.

Repeated CS+ presentation without the US (i.e., extinction) indicates that the CS+ no longer is a valid threat cue and that the CR should be adapted. This change in CS-US contingencies causes the formation of an extinction memory, which inhibits the original excitatory CS-US association and causes a decrease in the magnitude of the conditioned fear response (Quirk & Mueller, 2008). Importantly, only successful consolidation, retention, and recall of conditioning and extinction memories eventually lead to stable conditioned or extinguished fear responses (Myers & Davis, 2007; Quirk & Mueller, 2008).

Dopamine has been repeatedly associated with various learning processes including fear extinction (Abraham, Neve, & Lattal, 2014). Although both *within-session* extinction (i.e., decrement of the CR over the course of extinction training) and extinction *retention* (i.e., CR reductions to extinguished CSs in a delayed recall test) may involve prefrontal and dopaminergic mechanisms, they constitute different

* Corresponding author at: Department of Psychology, University of Marburg, Gutenbergstr. 18, 35032 Marburg, Germany.
E-mail addresses: christian.panitz@staff.uni-marburg.de (C. Panitz), matthias.sperl@staff.uni-marburg.de (M.F.J. Sperl), juergen.hennig@psychol.uni-giessen.de (J. Hennig), tim.klucken@uni-siegen.de (T. Klucken), christiane.hermann@psychol.uni-giessen.de (C. Hermann), erik.mueller@staff.uni-marburg.de (E.M. Mueller).

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neuropsychological processes with different dopaminergic networks involved (Myers & Davis, 2007; Vervliet, Craske, & Hermans, 2013). Of relevance, several rodent studies have shown impaired fear extinction retention – but not within-session extinction – following selective blockage of prefrontal dopamine receptors (Hikind & Maroun, 2008; Mueller, Bravo-Rivera, & Quirk, 2010; Pfeiffer & Fendt, 2006; but also see Ponnusamy, Nissim, & Barad, 2005).

In humans, individual differences in prefrontal dopamine are partially caused by the Val158Met single-nucleotide polymorphism on the catechol-O-methyltransferase gene (*COMT*). This gene controls activity of the COMT enzyme, which degrades extracellular catecholamines including dopamine. The Val variant of the *COMT* Val158Met polymorphism causes three- to fourfold COMT enzyme activity compared to the Met allele (Lachman et al., 1996; Weinshilboum, Otterness, & Szumlanski, 1999), causing a reduction in tonic dopamine levels, especially in the prefrontal cortex (PFC; Bilder, Volavka, Lachman, & Grace, 2004; Meyer-Lindenberg & Weinberger, 2006; Yavich, Forsberg, Karayiorgou, Gogos, & Mannisto, 2007). Its association with prefrontal dopamine levels makes *COMT* Val158Met an intriguing candidate polymorphism for modulation of fear extinction retention. Of relevance, a rodent study found reduced extinction retention in Met vs. Val homozygotes, as indicated by initially increased freezing to a previously extinguished CS+ during a recall test 24 h after extinction training (Risbrough, Ji, Hauger, & Zhou, 2014). Thus far, human COMT studies have focused on within-session extinction or fear retention (rather than extinction retention), albeit with mixed findings (Gruss, Langaee, & Keil, 2016; Klucken et al., 2016; Lonsdorf et al., 2009; Norrholm et al., 2013; Raczka et al., 2011). Given the putative role of prefrontal dopamine in *long-term fear extinction*, a human *COMT* Val158Met study with a delayed extinction recall test is warranted.

On the level of personality, differences in conditioning and extinction have been linked to traits such as neuroticism and extraversion (Eysenck, 1970; Zinbarg & Revelle, 1989). Neuroticism and closely linked anxiety (Depue & Lenzenweger, 2005) are strong predictors for the development of anxiety disorders (Clark, Watson, & Mineka, 1994). Neuroticism/anxiety is the most commonly investigated personality trait in fear conditioning research and higher levels of neuroticism/anxiety have been suggested to go along with enhanced fear conditioning and/or impaired fear extinction (Barlow, Ellard, Sauer-Zavala, Bullis, & Carl, 2014; Lonsdorf & Merz, 2017). However, empirical support is mixed with regard to the question which learning phase is sensitive (i.e., initial acquisition, extinction or consolidation/retention of conditioned and extinguished fear), whether higher neuroticism/anxiety predicts stronger or weaker CS discrimination, or if there are any robust relationships at all (Gazendam et al., 2015; Gazendam, Kamphuis, & Kindt, 2013; Grillon et al., 2006; Guimarães, Hellewell, Hensman, Wang, & Deakin, 1991; Joos, Vansteenwegen, & Hermans, 2012; Lommen, Engelhard, & van den Hout, 2010; Martínez et al., 2012; Otto et al., 2007; Pineles, Vogt, & Orr, 2009; Pitman & Orr, 1986; Rauch et al., 2005; Sehlmeyer et al., 2011; Staples-Bradley, Treanor, & Craske, 2018; Wiggert et al., 2017). This result pattern, along with theoretical considerations, suggests that neuroticism/anxiety may be an invalid predictor of fear responses in the laboratory. More precisely, neuroticism/anxiety is thought to predispose individuals to behavior which is (a) proactive to avoid more distant threats, (b) includes various risk assessment strategies (e.g., worrying, increased sensory intake) and (c) is most pronounced in ambiguous situations (Blanchard, Hynd, Minke, Minemoto, & Blanchard, 2001; McNaughton, 2011; Perkins & Corr, 2006). However, classical fear conditioning paradigms usually employ conditioned stimuli which reliably predict the occurrence of a clearly aversive stimulus only seconds later. Therefore, trait fearfulness might prove more relevant as it specifically describes the disposition for (a) intense reactive behavior in response to imminent threat, (b) including fighting, fleeing, or freezing and (c) which is most pronounced in unambiguously dangerous situations (Blanchard et al., 2001; McNaughton, 2011; Perkins & Corr, 2006). Previous studies have highlighted conceptual (Depue & Lenzenweger, 2005; McNaughton, 2011; Perkins & Corr, 2006; Perkins, Kemp, & Corr, 2007; Sylvers, Lilienfeld, & LaPrairie, 2011) as well as biological distinctions between neuroticism/anxiety and fearfulness (McNaughton & Corr, 2004; Walker & Davis, 2002; Walker, Toufexis, & Davis, 2003; White & Depue, 1999) that apparently are relevant in fear acquisition and short-term extinction (Gazendam et al., 2015). To our knowledge, no study has investigated the specific role of fearfulness in long-term fear extinction recall.

Meanwhile, high levels of extraversion have been associated with weaker conditioning and faster extinction learning (Eysenck, 1970). Similar to neuroticism/anxiety, the role of extraversion for *fear* conditioning and extinction has found only limited empirical support (Rauch et al., 2005) with several studies reporting mixed (Pineles et al., 2009) or null effects (Guimarães et al., 1991; Martínez et al., 2012; Otto et al., 2007). In the study of long-term fear extinction, however, the agency facet of extraversion (describing the disposition to be active, assertive, achievement-oriented) might be more predictive than general extraversion due to its putative dopaminergic basis (Depue & Collins, 1999; Wacker, Chavanon, & Stemmler, 2006; Wacker, Mueller, Hennig, & Stemmler, 2012).

The present study investigated if and in which manner (a) the *COMT* Val158Met polymorphism as well as (b) neuroticism/anxiety, (c) fearfulness and (d) agentic extraversion modulate fear and extinction retention in a delayed recall test. We employed an established two-day fear conditioning and extinction paradigm (Mueller, Panitz, Hermann, & Pizzagalli, 2014). On Day 1, two reinforced CS+ and two CS− were presented during an initial fear acquisition phase. In a subsequent extinction phase, one of the two CS+ and one of the two CS− were presented without a US. One day later, all four CS were presented again in a recall test to assess long-term recall of conditioned and extinguished fear. We assessed the Late Positive Potential (LPP) and fear bradycardia as cortical and autonomic components of the conditioned fear response, respectively. The LPP is a sustained positivity in the posterior event-related potential and is sensitive to motivational stimulus significance (Keil et al., 2002; Lang & Bradley, 2010). It has been used in previous fear conditioning studies on acquisition (Nelson, Weinberg, Pawluk, Gawlowska, & Proudfit, 2015) and extinction recall (Panitz, Hermann, & Mueller, 2015). Fear bradycardia describes cardiac slowing between 2 and 5 s after detection of a threat cue (Vila et al., 2007) and indicates focused attention as part of a freezing response (Lang, Davis, & Öhman, 2000; Löw, Lang, Smith, & Bradley, 2008; Löw, Weymar, & Hamm, 2015). It has been used to investigate fear acquisition (Bradley, Moulder, & Lang, 2005; Panitz et al., 2015; Sperl, Panitz, Hermann, & Mueller, 2016), extinction (Notterman, 1952; Panitz et al., 2015; Sperl et al., 2016) and long-term recall of conditioned fear (Panitz et al., 2015). In addition, we assessed skin conductance responses (SCR) and affective CS ratings as additional, widely used measures of conditioned fear (Lonsdorf et al., 2017).

2. Method

2.1. Sample and genotyping

$N = 383$ individuals recruited from campus and the community were initially screened for *COMT* Val158Met genotype. Buccal cell DNA was purified and genotyped using established protocols (Reuter & Hennig, 2005). The resulting genotype distribution ($n = 103$ Val/Val, $n = 196$ Val/Met, $n = 84$ Met/Met) did not deviate significantly from the Hardy-Weinberg equilibrium ($X^2(1) = 0.26$, $p = .611$).

All participants were right-handed males between 18 and 35 years. Exclusion criteria were habitual use of tobacco, psychotropic and illegal substances, body-mass index < 17 or > 30, as well as existing neurological, cardiovascular or psychopathological conditions. They were invited based on the *COMT* Val158Met polymorphism to create genotype-balanced groups. The investigated sample included $N = 93$

Table 1
Mean, SD and univariate ANOVA results for *COMT* Val158Met group differences in each personality compound measure.

	Mean (SD)			ANOVA	
	Val/Val	Val/Met	Met/Met	η^2	p
Fearfulness	1.62 (4.64)	0.08 (5.41)	−1.82 (5.12)	**.073**	**.041**
Neuroticism/Anxiety	0.00 (0.98)	0.12 (0.91)	−0.13 (0.76)	.013	.577
Agentic Extraversion	0.11 (0.86)	0.04 (0.73)	−0.16 (0.67)	.023	.377

participants (n = 32 Val/Val, n = 31 Val/Met, n = 30 Met/Met). One participant had to be excluded due to corrupted EEG data and five participants due to constant slow drift artifacts interfering with LPP measurement. Therefore, the final sample size was N = 87 (n = 30 Val/Val, n = 29 Val/Met, n = 28 Met/Met). Genotype groups did not differ in neuroticism/anxiety ($F(2, 84) = 0.55$, $p = .577$, $\eta_p^2 = .013$; for operationalization of personality, see next paragraph) or agentic extraversion ($F(2, 84) = 0.99$, $p = .377$, $\eta_p^2 = .023$). However, there appeared to be a (positive) dose effect of the Val allele on fearfulness ($F(2, 84) = 3.33$, $p = .041$, $\eta_p^2 = .073$; also, see Table 1 for genotype effects on compound scores of personality and Supplementary Table 1 for genotype effects on every single personality scale). The study protocol was in accordance to the Declaration of Helsinki and approved by the ethics committee of the German Psychological Association (DGPs).

2.2. Personality measures

Fearfulness. Fearfulness was measured with the Harmavoidance scale from the Multidimensional Personality Questionnaire (MPQ, Tellegen & Waller, 2008). The Harmavoidance scale contains several items asking participants for their preference between a dangerous/harmful and a boring event (e.g., 'receiving an electric shock' vs. 'waiting in line') and high values in this scale may reflect an individual's low sensation seeking tendencies, high fearfulness, or both. To account for this potential confound, we partialled out the Sensation Seeking scale of the Zuckerman-Kuhlman Personality Questionnaire (ZKPQ; Zuckerman, Kuhlman, Joireman, Teta, & Kraft, 1993) from the Harmavoidance scores to compute fearfulness scores.

Neuroticism/anxiety. Neuroticism/anxiety was measured with a compound score (averaged z-scores of each scale) of the German versions of the Neuroticism scale of the NEO Five Factor Inventory (Costa & McCrae, 1989), the Carver and White BIS scale (Carver & White, 1994), the ZKPQ Neuroticism-Anxiety scale, the MPQ Stress Reaction scale, and the Trait scale of the State-Trait Anxiety Inventory (Spielberger, Gorsuch, & Lushene, 1970). Computation of a compound score allowed us to achieve higher reliability of personality measures and to integrate information from different theoretical models while reducing multiple testing with very similar questionnaires (cf. Wacker et al., 2012). The inter-scale correlations were high ($.550 \leq r \leq .861$), justifying aggregation to increase measurement reliability.

Agentic extraversion. As for neuroticism/anxiety, we computed compound scores for agentic extraversion, averaging z-scores from the Carver and White BAS scale, the ZKPQ Activity scale, and the MPQ Social Potency scale. All inter-scale correlations were positive and significant ($.305 \leq r \leq .470$).

Compound scores for neuroticism/anxiety and agentic extraversion showed a small negative correlation ($r = -.240$, $p = .025$) but both were unrelated to fearfulness. Table 2 shows the inter-correlations between the different questionnaire scales and compound scores, respectively.

2.3. Conditioning and extinction procedures

Paradigm. Participants underwent a two-day differential fear conditioning and extinction paradigm similar to Mueller et al. (2014). On Day 1, the paradigm started with a habituation phase in which each of four CS was presented five times. The following acquisition phase consisted of three blocks, each with 15 presentations per CS resulting in a total of $3 \times 15 \times 4 = 180$ CS presentations. Stimulus order was randomized. Two of the CS (CS + E, CS + N) were paired with the US in 7 out of 15 trials per block (46.6% reinforcement rate), the other two CS (CS − E, CS − N) were never paired with the US. After the acquisition phase, participants received a standardized breakfast, completed a series of questionnaires and were prepared for the following extinction phase. The extinction phase started exactly three hours after the end of acquisition and consisted of a single block of 40 CS + E and CS − E trials each ("E" standing for *presented during extinction phase*). CS + N, CS − N, and US were not presented during this phase ("N" standing for *not presented during extinction phase*). The recall test on Day 2 consisted of three blocks with 20 trials of each CS, no US were presented (paradigm depicted in Fig. 1).

Stimuli and trial structure. Four male faces with neutral expression from the Karolinska Directed Emotional Faces series (Lundqvist, Flykt, & Öhman, 1998) were used as CS (pictures: AM10NES, AM13NES, AM31NES, BM08NES, also see Fig. 1). Assignment of face stimuli to the different CS types was permutated and balanced across participants. As better suitability of white noise vs. electric shock during fear conditioning with many trials has previously been demonstrated (Sperl et al., 2016), the US was a 1 s white noise burst delivered by a room speaker at 95 dB(A) (measured at the participant's head position, 2.30 m from the speaker). In every trial, a fixation cross (1 s duration) was presented before participants saw the CS for 4 s. In reinforced trials the CS co-terminated with the 1 s US. A black screen (jittered, 6–8 s) was presented between trials.

2.4. Affective ratings

Participants rated each CS on a 5-point Likert scale for pleasantness (1 = "very unpleasant", 5 = "very pleasant") and arousal (1 = "not arousing", 5 = "very arousing"). Ratings were assessed before and after every conditioning phase as well as between blocks. Directly before and after Day 1 extinction, only CS + E and CS − E were rated. Pleasantness ratings were reversed into unpleasantness scores before statistical analyses.

2.5. Physiological recording and processing

All electrophysiological signals were recorded at 1000 Hz using a QuickAmp 72 amplifier (Brain Products, Gilching, Germany). Marker latencies were corrected for monitor delay (33 ms; assessed with a Brain Products photo sensor).

EEG and ERP. 64-channel EEG was recorded during extinction and recall test using actiCAP active electrodes (Brain Products, Gilching, Germany), with a 200 Hz online lowpass-filter and referenced against the average. EEG processing was performed in BrainVision Analyzer 2 (Brain Products, Gilching, Germany). The EEG was downsampled to 500 Hz, highpass-filtered (−3 dB at 0.1 Hz, 24 dB/oct., zero-phase IIR Butterworth filter; same specifications for all other high- and lowpass filtering procedures) and notch-filtered (50 Hz, 5 Hz bandwidth, 16th order). Blinks and oculomotor artifacts were removed using extended Infomax ICA (Lee, Girolami, & Sejnowski, 1999). Critical components were identified based on signal shape and topography of ICA weights by an experienced rater. To increase signal stationarity required for ICA, large EEG artifacts were removed manually, the signal was 0.5 Hz highpass-filtered for ICA only and the resulting weights were subsequently applied to the 0.1 Hz filtered data (Winkler, Debener, Müller, & Tangermann, 2015). Data with remaining artifacts was removed manually. Channels with excessive amounts of bad data were interpolated (spline). Finally, EEG data were lowpass-filtered (30 Hz), segmented relative to CS onset (−200 to 1000 ms), baseline-corrected, averaged across trials and referenced against the average of TP9 and

Table 2
Correlations between questionnaire scales and between compound scores.

	Fearfulness		*Neuroticism/Anxiety*					*Agentic Extraversion*		
	HA	*SS*	*N*	*NAnx*	*SR*	*BIS*	*STAI-T*	*Act*	*SP*	*BAS*
HA	**.852**									
SS	-.390***	**.777**								
N	-.001	-.202	**.820**							
NAnx	.065	-.151	.743***	**.827**						
SR	.087	-.189	.795***	.861***	**.861**					
BIS	.251*	-.278**	.671***	.660***	.649***	**.848**				
STAI-T	.009	-.214*	.831***	.753***	.769***	.550***	**.917**			
Act	-168	.263*	-.156	-.186	-.049	-.249*	-.211*	**.778**		
SP	-.167	.412***	-.243*	-.183	-.191	-.328**	-.252*	.470***	**.896**	
BAS	-.138	.368***	-.077	-.054	-.043	.060	-.268*	.305**	.334**	**.811**

	Fearfulness	*Neuroticism/Anxiety (N/A)*	*Agentic Extraversion (agE)*
Fearfulness	**.829**		
N/A	.002	**.897**	
agE	-.031	-.240*	**.608**

Note: $^{*}p < .05$, $^{**}p < .01$, $^{***}p < .001$, two-sided. Cronbach's alpha for each scale printed in bold. Gray-shaded areas contain inter-correlations of scales belonging to the same compound score. Cronbach's alpha for Fearfulness was computed after partialling out the Sensation Seeking sum score from the single Harmavoidance items. Cronbach's alpha for Neuroticism/Anxiety and Agentic Extraversion were computed with questionnaire scores as items. HA = MPQ Harmavoidance; SS = ZKPQ Sensation Seeking; N = NEO-FFI Neuroticism; NAnx = ZKPQ-Neuroticism/Anxiety; SR = MPQ Stress Reaction; BIS = Carver & White BIS; STAI-T = STAI-Trait; Act = ZKPQ Activity; SP = MPQ Social Potency; BAS = Carver & White BAS. Fearfulness = HA controlled for SS; N/A(Neuroticism/Anxiety) = mean z-standardized scores of N, NAnx, SR, BIS, STAI-T; agE (Agentic Extraversion) = mean z-standardized scores of Act, SP, BAS.

TP10 (mastoids).

Fear bradycardia. ECG was measured during all phases in a lead II configuration with bipolar measurement electrodes on the left leg and right forearm and the ground electrode on the left forearm. ECG data was bandpass-filtered in BrainVision Analyzer 2 (1–30 Hz, filter type identical with EEG filters). R spikes were detected automatically with the ECG Markers solution implemented in the Analyzer software and corrected manually if necessary. ECG data was rejected when it contained a ventricular extrasystole or artifacts that prevented unambiguous R spike detection. Eventually, a continuous heart period (HP) trace was computed with every sample point reflecting the distance between the preceding and the subsequent R spike in milliseconds. The HP signal then was segmented relative to CS onset (−1–8 s), baseline-corrected and averaged across trials. The resulting signal was divided into 500 ms time bins by averaging the magnitude of all 500 data points within each time window. Because US presentation (3–4 s relative to CS onset) fell into the segmentation window of reinforced CS+ presentations, only non-reinforced trials were analyzed for the acquisition phase.

SCR. Skin conductance was collected during all phases with exosomatic measurement (0.5 V DC) on the left hand's thenar and hypothenar. The signal was lowpass-filtered at 1 Hz (filter type identical with EEG), manually screened for artifacts and segmented relative to CS (−1–5 s). Using a custom script in MATLAB 2013a (Mathworks, Natick, MA, USA), segments were baseline-corrected before single-trial peak values (time window: 1–5 s) were automatically scored, averaged across trials and normalized according to Lykken & Venables (1971). Resulting SCR values were log-transformed to approximate normality

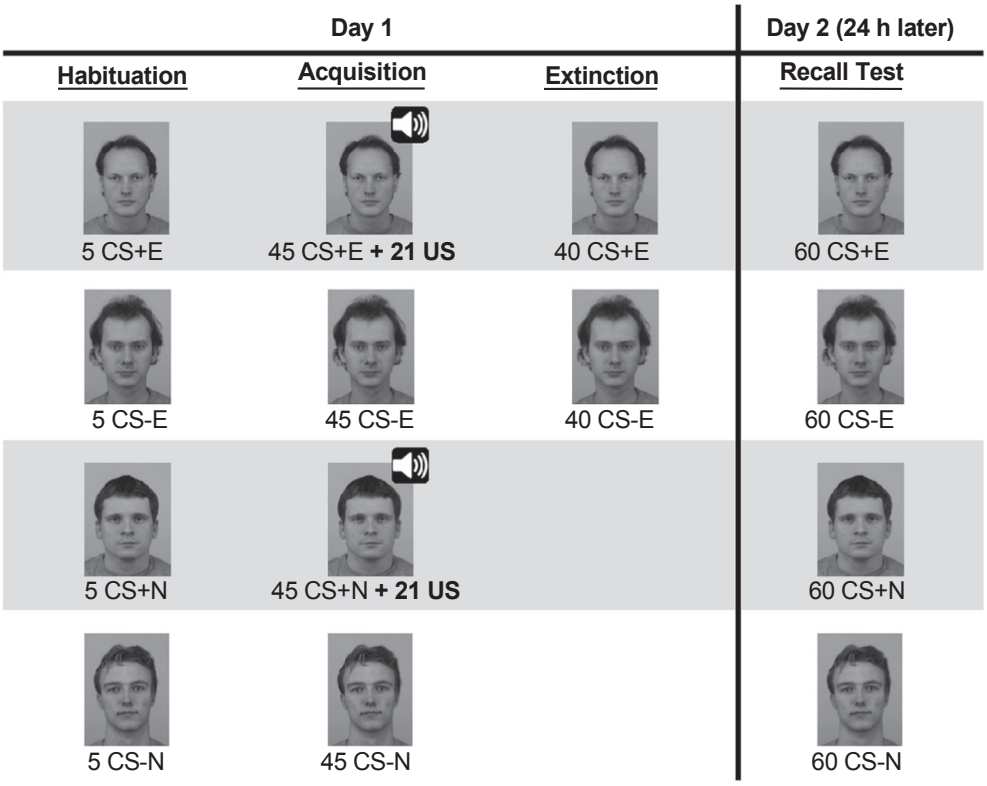

Fig. 1. Learning phases. Face stimuli and number of presentations in the two-day differential fear conditioning and extinction paradigm. US was only presented during acquisition phase, indicated by the speaker symbol. Assignment of different faces to CS type was permutated across participants. CS + E = extinguished CS+, CS + N = non-extinguished CS+, CS − E = CS− presented during extinction phase, CS − N = CS− not presented during extinction phase. Stimuli were presented in color. KDEF stimuli IDs from top to bottom: AM10NES, AM13NES, AM31NES, BM08NES.

(LN(1 + SCR)). Only non-reinforced trials were analyzed for the acquisition phase.

2.6. Statistical analyses

We computed ANOVAs on affective ratings, fear bradycardia and SCR for all three phases and on LPP for Day 1 extinction training and Day 2 recall test. For both Day 1 fear acquisition and Day 2 recall test, Contingency (CS + E/N vs. CS − E/N) × Extinction (CS ± E vs. CS ± N) × Genotype (Val/Val vs. Val/Met vs. Met/Met) ANOVAs were conducted. For Day 1 extinction training, Contingency (CS + E vs. CS − E) × Genotype (Val/Val vs. Val/Met vs. Met/Met) ANOVAs were computed. The factor Extinction was not included in the analysis because CS + N and CS − N were not presented during extinction phase.

In addition to the analyses across all trials, we computed ANOVAs for LPP, HP and SCR on Day 1 with the additional factor Time (first ten artifact-free trials vs. last ten artifact-free trials) in order to assess short-lasting, transient effects during ongoing learning (Gruss et al., 2016; Risbrough et al., 2014; Sperl et al., 2016). Following the same logic, we conducted all Day 2 recall test ANOVAs again using only the first ten artifact-free trials in order to assess *initial* long-term recall. We used an identical design for ANOVAs on unpleasantness and arousal ratings: for the acquisition and extinction phases we entered the ratings at the beginning and at the end of each phase, respectively. For Day 2 recall test we used the ratings at the beginning as they indicate initial recall.

To assess the role of personality, we computed linear regression analyses for the differential responses to CS+ vs. CS− in Day 1 acquisition, Day 1 extinction, and Day 2 recall as well as for the long-term extinction contrast ([CS + N − CS − N] − [CS + E − CS − E]) in Day 2 recall. For SCR, fear bradycardia, and LPP we used the CR across all trials, for unpleasantness and arousal we used ratings after acquisition, after extinction and before recall test. In every analysis, all personality measures (i.e., fearfulness, neuroticism/anxiety, agentic extraversion) were entered simultaneously. In addition to the regression analyses, we computed ANCOVAs with the factors Contingency, Extinction, and Genotype and entered Fearfulness, Neuroticism/Anxiety and Agentic Extraversion as centered covariates. Thereby, we were able to rule out that associations between personality measures and contrasts of interest can be explained (a) by higher-/lower-order effects or (b) by genotype as a common basis. Overall, ANCOVA results confirmed regression analyses and are reported in the supplementary material. Moreover, in order to facilitate comparability with other studies, we also provide bivariate correlations between single questionnaire measures and conditioned response contrasts in Supplementary Table 2.

3. Results

3.1. Acquisition

Affective ratings. Indicating successful conditioning at the level of self-reported affect, there were main effects of Contingency for unpleasantness and arousal ratings, with CS+ rated as more unpleasant ($F(1, 84) = 31.99$, $p < .001$, $\eta_p^2 = .276$) and more arousing ($F(1, 84) = 26.69$, $p < .001$, $\eta_p^2 = .241$) than CS−. These main effects were essentially caused by Contingency × Time interactions (unpleasantness: $F(1, 84) = 35.32$, $p < .001$, $\eta_p^2 = .296$; arousal: $F(1, 84) = 28.66$, $p < .001$, $\eta_p^2 = .254$), as CS+ vs. CS− ratings differed after acquisition (unpleasantness: $F(1, 84) = 42.68$, $p < .001$, $\eta_p^2 = .337$, Fig. 2a); arousal: $F(1, 84) = 28.66$, $p < .001$, $\eta_p^2 = .254$, Supplementary Figure 1b) but not before (unpleasantness: $F(1, 84) = 0.51$, $p = .479$, $\eta_p^2 = .006$; arousal: $F(1, 84) = 1.01$, $p = .301$, $\eta_p^2 = .013$). There were no effects of Genotype or other factors (all $p > .088$, $\eta_p^2 < .057$). With regard to personality measures, none of the full regression models reached significance (both $R^2 < .061$, $p > .158$), although higher fearfulness predicted higher unpleasantness ratings for CS+ vs. CS− ($\beta = .244$, $p = .025$) at the end of acquisition (Fig. 2b). See Supplementary Tables 2–4 for bivariate correlations of single questionnaires and ANCOVA results.

Fear bradycardia. Converging with the subjective ratings, fear conditioned stimuli successfully evoked fear bradycardia, i.e., HP slowing was stronger for CS+ than for CS−. This was evident in a main effect of Contingency ($F(1, 84) = 32.08$, $p < .001$, $\eta_p^2 = .281$, Fig. 2c,

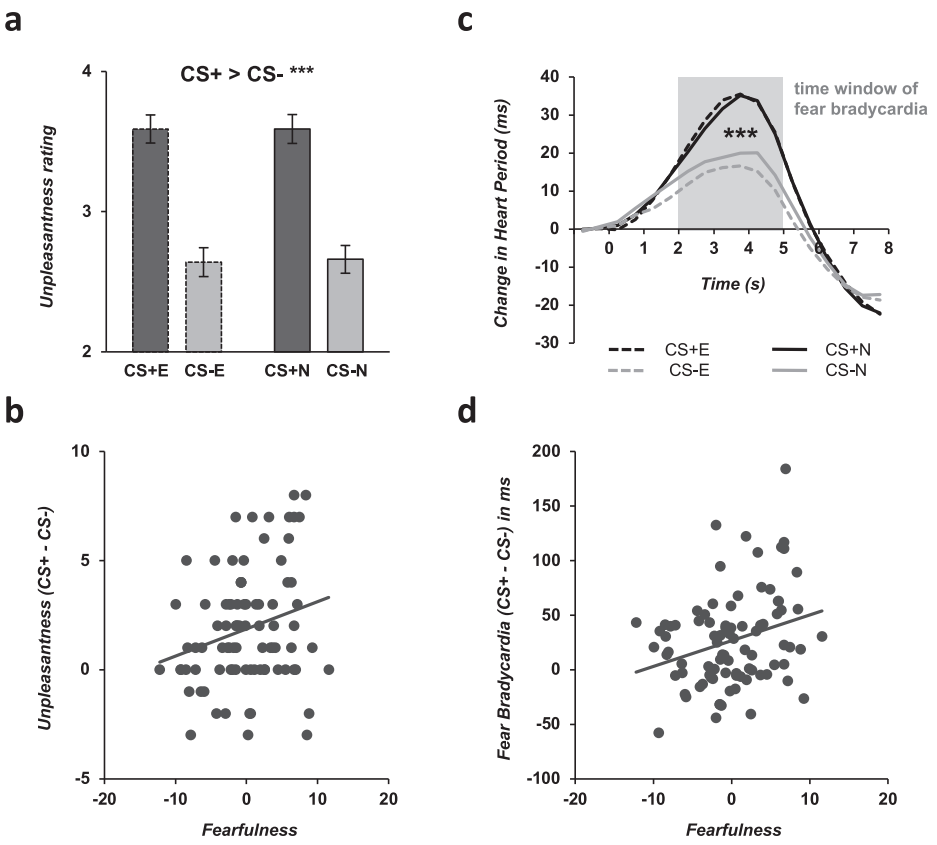

Fig. 2. Conditioning effects on unpleasantness ratings, fear bradycardia and their relationship with fearfulness. (a) Mean unpleasantness ratings at the end of fear acquisition phase. Error bars indicate SEMs, based on within-subject variance. *** $p < .001$ for main effect of Contingency. CS+E = extinguished CS+, CS+N = non-extinguished CS+, CS−E = CS− presented during extinction phase, CS−N = CS− not presented during extinction phase. (b) Relationship between fearfulness and differential unpleasantness ratings (both CS+ vs. both CS−) at the end of fear acquisition. (c) Change in heart period relative to baseline during fear acquisition phase. Mean magnitude within the shaded area (2–5 s post-CS) was used for statistical analyses of fear bradycardia. CS+E = extinguished CS+, CS+N = non-extinguished CS+, CS−E = CS− presented during extinction phase, CS−N = CS− not presented during extinction phase. (d) Relationship between fearfulness and differential fear bradycardia (both CS+ vs. both CS−) during fear acquisition.

Supplementary Figure 2b). There also was an unexpected Genotype × Extinction interaction ($F(2, 84) = 3.67$, $p = .031$, $\eta_p^2 = .080$) due to stronger HP slowing for the (to-be) non-extinguished stimuli compared to extinguished stimuli (i.e., [CS+N+CS−N] > [CS+E+CS−E]) in Met/Met carriers ($p_{Bonferroni} = .030$). The additional analysis of the first and last ten trials, that was performed to probe within-session dynamics, revealed a general increase in HP slowing over time (main effect Time, $F(1, 84) = 5.51$, $p = .021$, $\eta_p^2 = .062$), and, again, a strong main effect of Contingency ($F(1, 84) = 45.93$, $p < .001$, $\eta_p^2 = .353$). There were no significant effects of Genotype or any other significant effects (all $p > .150$, $\eta_p^2 < .045$).

When testing the effects of the three investigated personality traits the full regression model was significant ($R^2 = .091$, $F(3, 83) = 2.75$, $p = .046$) and fearfulness was positively associated with differential fear bradycardia ($\beta = .286$, $p = .008$; Fig. 2d; Table 3). Furthermore, follow-up analyses revealed that more fearful individuals showed increased CRs specifically to CS+ ($\beta = .255$, $p = .017$) but not CS− ($\beta = .068$, $p = .537$; Table 3). See Supplementary Tables 2 and 5 for bivariate correlations of single questionnaires and ANCOVA results.

SCR. In line with the fear bradycardia findings, CS+ evoked a stronger SCR than CS− as evident in a main effect of Contingency ($F(1, 84) = 27.64$, $p < .001$, $\eta_p^2 = .248$, Supplementary Figure 2a). There were no effects of Genotype (all $p > .099$, $\eta_p^2 < .054$) or personality traits ($R^2 = .027$, $p = .515$). When analyzing only the first and last trials of the acquisition phase, a general SCR habituation over time emerged (main effect Time, $F(1, 84)$, $p < .001$, $\eta_p^2 = .211$) and the main effect of Contingency was confirmed ($F(1, 84)$, $p < .001$, $\eta_p^2 = .163$). See Supplementary Tables 2 and 6 for bivariate correlations of single questionnaires and ANCOVA results.

Table 3
β weights for regression of Day 1 acquisition heart period on personality compound measures.

	Day 1 acquisition – fear bradycardia across all trials		
	diff CR	CS+	CS−
Fearfulness	**.286****	**.255***	.068
Neuroticism/Anxiety	.012	.009	.001
Agentic Extraversion	.108	.158	.098

Note: diff CR = differential conditioned response (i.e., CS+ − CS−).
* $p < .05$.
** $p < .01$.

3.2. Extinction

Affective ratings. ANOVAs revealed significant main effects of Contingency, as CS+E were rated both as more unpleasant ($F(1, 84) = 8.36$, $p = .005$, $\eta_p^2 = .091$) and more arousing ($F(1, 84) = 8.41$, $p = .005$, $\eta_p^2 = .091$) than CS−E at pre- and post-extinction (also see Supplementary Figures 1a and 1b). A main effect of Time indicated that participants rated both CS as less unpleasant ($F(1, 84) = 17.97$, $p < .001$, $\eta_p^2 = .176$) and arousing ($F(1, 84) = 9.49$, $p = .003$, $\eta_p^2 = .101$) at the end of extinction. No further significant effects emerged for unpleasantness (all $p > .121$, $\eta_p^2 < .029$) or arousal (all $p > .310$, $\eta_p^2 < .028$), including any Genotype effects. Personality measures did not predict affective ratings after extinction (both $R^2 < .015$, $p > .749$). See Supplementary Tables 2, 7 and 8 for bivariate correlations of single questionnaires and ANCOVA results.

Fear bradycardia. Consistent with successful conditioning recall in the extinction phase (which took place three hours after acquisition), HP slowing remained to be stronger for CS+E than for CS−E (main effect Contingency, $F(1, 84) = 10.52$, $p = .002$, $\eta_p^2 = .111$, Supplementary Figures 2b and 3). No other effects reached significance (all $p > .230$, $\eta_p^2 < .035$). When analyzing only the first and last ten trials, there was a general reduction in HP over the extinction phase (main effect Time, $F(1, 84)$, $p < .001$, $\eta_p^2 = .202$) but no other significant effects, including Genotype effects (all $p > .123$, $\eta_p^2 < .029$). Personality measures did not predict differential responses (CS+E –

CS−E) during Day 1 extinction (R^2 = .033, p = .422). See Supplementary Tables 2 and 9 for bivariate correlations of single questionnaires and ANCOVA results.

SCR. In line with the fear bradycardia results, CS+E evoked a stronger SCR than CS−E (main effect Contingency, $F(1, 84)$ = 5.72, p = .019, η_p^2 = .064, Supplementary Figure 2a) across all trials. There were no other significant effects or relationships with Genotype (all p > .055, η_p^2 < .067) or personality (R^2 = .044, p = .284). When only the first and last trials were analyzed, there was a general habituation of the SCR over time (main effect Time, $F(1, 84)$ = 29.23, p < .001, η_p^2 = .258) and a trend for the main effect Contingency remained ($F(1, 84)$ = 3.32, p = .072, η_p^2 = .038). No other significant effects were found (all p > .231, η_p^2 < .035). See Supplementary Tables 2 and 10 for bivariate correlations of single questionnaires and ANCOVA results.

LPP. Converging with HP and SCR and as predicted from prior studies (Nelson et al., 2015; Panitz et al., 2015), the LPP amplitude was increased for CS+E vs. CS−E ($F(1, 84)$ = 3.26, $p_{one\text{-}sided}$ = .037, η_p^2 = .037; Supplementary Figure 2c). There were no significant genotype-related effects (both p > .116, η_p^2 < .050). When analyzing only the first and last ten trials, no significant effects emerged (all p > .204, η_p^2 < .026). Personality measures did not predict differential LPP responses (CS+E – CS−E) during Day 1 extinction (R^2 = .030, p = .464). See Supplementary Tables 2 and 11 for bivariate correlations of single questionnaires and ANCOVA results.

3.3. Recall test

Affective ratings. At the beginning of Day 2 recall test, participants rated CS+ to be more unpleasant (main effect Contingency, $F(1, 84)$ = 9.45, p = .003, η_p^2 = .101, Supplementary Figure 1a) and arousing (main effect Contingency, $F(1, 84)$ = 8.20, p = .005, η_p^2 = .089, Supplementary Figure 1b) than CS−. No other effects emerged for unpleasantness (all p > .108, η_p^2 > .052) or arousal (p > .244, η_p^2 = .033). Personality did not predict contingency effects (CS+ vs. CS−; both R^2 < .026, p > .539) nor differential extinction recall ([CS+N vs. CS−N] – [CS+E – CS−E]; both R^2 < .051, p > .228). See Supplementary Tables 2, 12 and 13 for bivariate correlations of single questionnaires and ANCOVA results.

Fear bradycardia. During the Day 2 recall test CS+ continued to evoke stronger cardiac slowing than CS− when trials were averaged across the entire recall session (main effect of Contingency, $F(1, 84)$ = 7.40, p = .007, η_p^2 = .081, Supplementary Figures 2b and 3), converging with Day 2 conditioned fear bradycardia observed in a previous study (Panitz et al., 2015). No other effects reached significance (all p > .355, η_p^2 < .023).

Importantly, at the beginning of the Day 2 recall test (i.e., first ten artifact-free trials), there was a Genotype × Contingency × Extinction interaction $F(2, 84)$ = 4.66, p = .012, η_p^2 = .100). Follow-up two-way interaction contrasts revealed that only Val/Val ($F(1, 29)$ = 11.28, p = .002, η_p^2 = .280) but neither Val/Met nor Met/Met (both p > .143, η_p^2 < .078) carriers showed a significant Contingency × Extinction interaction. Consistent with successful fear and extinction recall, the significant interaction in Val/Val carriers was characterized by a reduced differential response to extinguished (CS+E – CS−E) compared to non-extinguished (CS+N – CS−N) stimuli (see Fig. 3). The interaction contrast (i.e., [CS+N − CS−N] − [CS+E − CS−E]) was larger in Val/Val carriers compared to Val/Met carriers ($t(57)$ = 2.40, p = .020, d = 0.62), mainly driven by a descriptively smaller extinguished fear response (CS+E – CS−E) in Val/Val vs. Val/Met carriers, indicating better extinction recall for the first group ($t(57)$ = −1.85, p = .070, d = −0.48). In addition, the interaction contrast was larger in Val/Val carriers compared to Met/Met carriers ($t(56)$ = 3.00, p = .004, d = 0.78), mainly driven by a significantly larger non-extinguished fear response (CS+N – CS−N) in Val/Val vs. Met/Met carriers, indicating stronger fear recall in the first group ($t(56)$ = 2.66, p = .010, d = 0.70). Meanwhile, the interaction contrast did not differ significantly between Val/Met and Met/Met carriers (p = .449, d = 0.20).

Personality measures did not predict differential fear responses (CS+ vs. CS−; R^2 = .017, p = .706), nor differential Day 2 extinction recall ([CS+N – CS−N] vs. [CS+E – CS−E]; R^2 = .040, p = .331). See Supplementary Tables 2 and 14 for bivariate correlations of single questionnaires and ANCOVA results.

SCR. As for fear bradycardia, faces that had been paired with the US on Day 1 (CS+) evoked a stronger SCR than never-paired faces (CS−; main effect of Contingency: $F(1, 84)$ = 5.72, p = .006, η_p^2 = .085, Supplementary Figure 2a). Replicating Mueller et al. (2014), the Contingency × Extinction interaction was significant for a one-sided test ($F(1, 84)$ = 3.59, $p_{one\text{-}sided}$ = .031, η_p^2 = .041) and indicated successful extinction recall with the differential fear reaction to CS+ vs. CS− being stronger for non-extinguished (η_p^2 = .105, p = .002) vs. extinguished CS (η_p^2 = .011, p = .347). All other effects were non-significant (all p > .129, p. η^2 < .048). When only the first ten artefact-free trials were analyzed (i.e., *initial* Day 2 recall), the main effect of Contingency had a descriptively weaker effect size (p = .068, η_p^2 = .039, two-sided). The Contingency × Extinction interaction did not reach significance in this analysis (p = .357, η_p^2 = .010). Personality measures did not predict differential fear responses (CS+vs. CS−; R^2 = .024, p = .569), nor differential extinction recall ([CS+N – CS−N] vs. [CS+E – CS−E]; R^2 = .018, p = .685). See Supplementary Tables 2 and 15 for bivariate correlations of single questionnaires and ANCOVA results.

LPP. The LPP across the entire recall session was modulated by a significant Genotype × Contingency × Extinction interaction ($F(2, 84)$ = 3.41, p = .038, η_p^2 = .075). Similar to the HP, two-way interaction contrasts within each genotype group revealed a significant Contingency × Extinction interaction that was restricted to Val/Val ($F(1, 29)$ = 6.04, p = .020, η_p^2 = .172) and absent in Val/Met or Met/Met carriers (both p > .229, η_p^2 < .054). Mirroring the HP analyses, the significant interaction in Val/Val carriers was caused by a smaller differential response for extinguished CS (CS+E – CS−E) compared to the non-extinguished response (CS+N – CS−N, Fig. 4). When comparing genotype groups, there was a larger Contingency × Extinction interaction effect in Val/Val compared to Met/Met carriers ($t(56)$ = 2.47, p = .017, d = 0.65), mainly driven by a significantly larger non-extinguished fear response (CS+N – CS−N) in Val/Val vs. Met/Met carriers, indicating stronger fear recall in the first group ($t(56)$ = 2.67, p = .010, d = 0.70). Although there were no differences for Val/Val vs. Val/Met and Val/Met vs. Met/Met carriers in the interaction contrast (both p > .130, d < 0.40), Val/Val carriers showed a stronger non-extinguished fear response in comparison to Val/Met carriers ($t(57)$ = 2.25, p = .029, d = 0.59). No other effects of the three-way ANOVA across all trials reached significance (all p > .225, η_p^2 < .035).

When only the *initial* recall (i.e., first ten artifact-free trials) was analyzed, there was a higher LPP amplitude for CS+ relative to CS− (main effect Contingency, $F(1, 84)$ = 7.62, p = .007, η_p^2 = .083). The three-way Genotype × Contingency × Extinction interaction did not reach significance ($F(2, 84)$ = 1.44, p = .243, η_p^2 = .033). Exploratory analyses within genotype groups revealed that again, the Contingency × Extinction interaction contrast was only significant in the Val/Val ($F(1, 29)$ = 7.33, p = .011, η_p^2 = .202) but not in the Val/Met and Met/Met groups (both p > .275, η_p^2 < .044). Similar to analyses across all trials, the non-extinguished fear response in the LPP (CS+N – CS−N) was larger in Val/Val carriers compared to Val/Met carriers ($t(57)$ = 2.38, p = .021, d = 0.62). Moreover, the main effect Contingency emerged within Val/Val (p = .024, η_p^2 = .164) and Met/Met (p = .049, η_p^2 = .136), but not Val/Met carriers (p = .938, η_p^2 = .000). No other significant effects emerged (all p > .068, η_p^2 < .040). Taken together, HP and LPP indicated successful recall of both conditioned and extinguished fear in Val/Val but not Met carriers.

Personality measures did not predict differential fear responses (CS+ vs. CS−; R^2 = .058, p = .175), nor differential Day 2 extinction

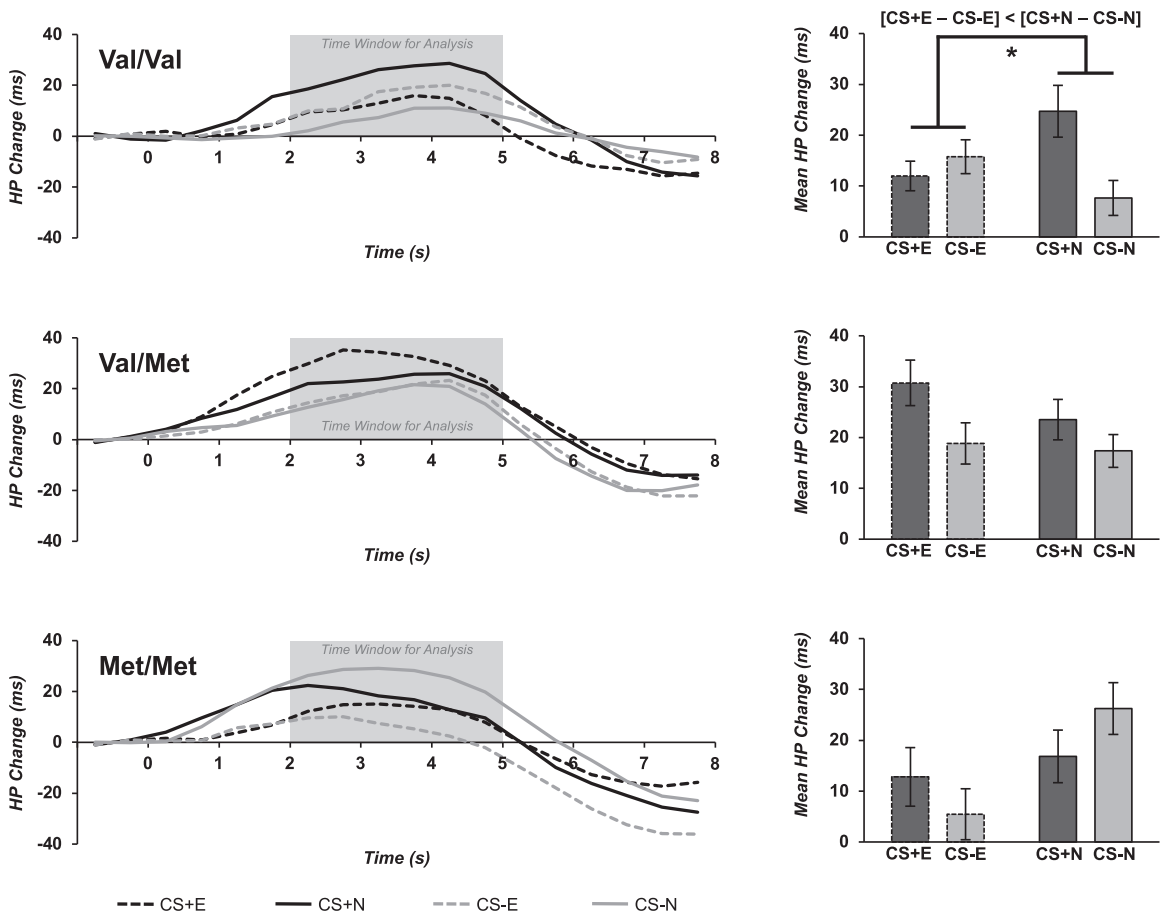

Fig. 3. *COMT* Val158Met effects on Day 2 fear bradycardia. CS-evoked change in heart period for the different genotype groups during the first ten artifact-free trials of Day 2 recall test. CS + E = extinguished CS+, CS + N = non-extinguished CS+, CS − E = CS− presented during extinction phase, CS − N = CS− not presented during extinction phase. Mean magnitude within the gray box was used for statistical analyses. Error bars in the bar plots indicate SEM based on within-subject variance. *Contingency × Extinction interaction ($p < .05$).

recall ([CS + N – CS − N] vs. [CS + E – CS − E]; $R^2 = .055$, $p = .191$). See Supplementary Tables 2 and 16 for bivariate correlations of single questionnaires and ANCOVA results.

3.4. Exploratory analyses on Day-1-Day-2 stability

As can be seen in Fig. 3, there appeared to be remaining Day 2 fear bradycardia to extinguished stimuli (i.e., CS + E > CS − E) in Met carriers (i.e., Val/Met and Met/Met carriers), although this effect was not statistically significant. We further observed significantly increased variance in extinguished fear bradycardia in Val/Met vs. Val/Val carriers (Levene test: $F(1, 57) = 6.83$, $p = .011$) and Met/Met vs. Val/Val carriers ($F(1, 56) = 5.13$, $p = .027$). This shifted our focus to individual differences within the Met carriers as it suggests that there may be a subgroup of Met carriers with particularly reduced extinction recall. We reasoned that, first of all, individual differences in Day 2 extinction recall (CS + E vs. CS − E) may be related to individual differences in Day 1 fear extinction success (e.g., Hermans et al., 2005). To test this, we correlated the fear response at the end of Day 1 extinction (CS + E – CS − E during the last ten trials) with the extinguished fear response at the beginning of Day 2 recall (CS + E – CS − E during the first ten trials). This correlation was positive and significantly different from zero within the entire sample ($r(85) = .222$, $p = .039$) indicating that successful extinction recall on Day 2 depended on successful extinction on Day 1. We then reasoned that this relationship may be more pronounced in Met carriers, thereby causing the high interindividual variance of Day 2 extinction recall. As expected, the Day-1-Day-2-correlation significantly differed between genotype groups ($F(2, 81) = 6.38$, $p = .033$, $\eta_p^2 = .081$). More specifically, both Val/Met carriers ($r(27) = .345$, $p = .067$) and Met/Met carriers ($r(26) = .400$, $p = .035$) showed a correlation between final Day 1 response and initial Day 2 response to extinguished CS while this correlation was absent in Val/Val carriers ($r(28) = -.222$, $p = .238$).

Associations with personality measures. To further test whether the fear bradycardia association between Day 1 fear extinction and Day 2 fear extinction recall (i.e., extinction stability) was moderated by personality we conducted a multiple regression analysis (criterion: initial Day 2 extinguished fear response; predictors: final Day 1 extinction fear response, personality measures, and for each personality measure its interaction with the Day 1 response). The regression model reached significance ($R^2 = .162$, $F(7, 79) = 2.24$, $p = .040$). In this analysis, the interaction of Day 1 fear bradycardia and neuroticism/anxiety was significant and negative ($\beta = -.310$, $p = .009$; Table 4). This interaction indicates that the association of Day 1 extinction and Day 2 extinction recall decreased with increasing neuroticism/anxiety (Supplementary Figure 4; also see Supplementary Table 17 for results of the different neuroticism/anxiety scales). Thus, low neurotic, little anxious, and emotionally stable individuals showed stable reactions to the extinguished fear stimuli on Day 1 and Day 2, while more anxious individuals appeared to not recall well what they had learned by the end of the Day 1 extinction session.

3.5. Power analyses

Using G*Power 3.1 (Faul, Erdfelder, Lang, & Buchner, 2007), we conducted post-hoc power analyses to estimate statistical power of the

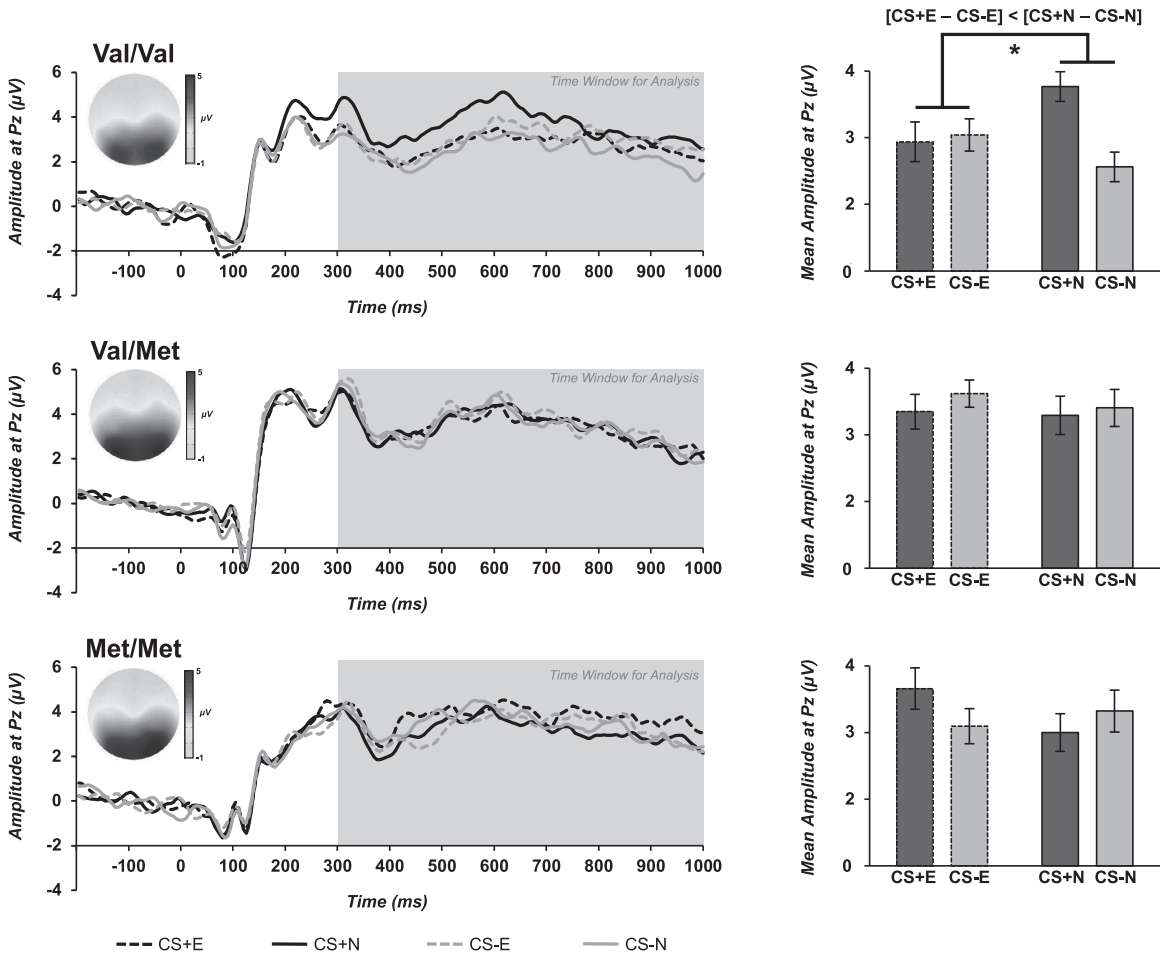

Fig. 4. *COMT* Val158Met effects on Day 2 LPP. ERPs and mean LPP amplitudes at Pz during Day 2 recall test for the different genotype groups. LPP was defined as the mean amplitude from 300 to 1000 ms (indicated by shaded areas). Topography plots show mean LPP amplitudes across all four CS. Error bars in the bar plots indicate SEM based on within-subject variance. CS+E = extinguished CS+, CS+N = non-extinguished CS+, CS−E = CS− presented during extinction phase, CS−N = CS− not presented during extinction phase. *Contingency × Extinction interaction ($p < .05$).

Table 4
β Weights for regression of initial Day 2 recall of extinguished heart period CR (CS+E – CS−E) on final Day 1 CR and personality compound measures.

	β
Day 1 CR	**.219***
Fearfulness	.122
Neuroticism/Anxiety	.037
Agentic extraversion	.095
Day 1 CR × Fearfulness	−.068
Day 1 CR × Neuroticism/Anxiety	**−.310****
Day 1 CR × Agentic extraversion	−.070

Note: Day 1 CR = differential fear bradycardia (CS+E − CS−E) during last ten extinction trials; All seven predictors were entered simultaneously.
* $p < .05$.
** $p < .01$.

present significant genotype and personality effects of interest ($\alpha = .05$) and a priori power analyses to estimate necessary sample sizes for replication with sufficient power ($1 - \beta = .80$). Most effects in fear bradycardia (i.e., Day 1 association with fearfulness, Day 2 Genotype × Contingency × Extinction interaction, Neuroticism/Anxiety effect on Day-1-Day-2 stability) had sufficient statistical power ($1 - \beta > .77$, two-sided tests). These effects could be replicated in a sample of comparable size ($N = 93$, two-sided test) or in even smaller samples when testing regression effects one-sided ($N = 71$). Meanwhile, the other three effects (i.e., Day 1 association of fearfulness and unpleasantness ratings, Day 2 Genotype × Contingency × Extinction interaction in the LPP, Genotype effects on Day-1-Day-2 stability) had smaller post-hoc power estimates ($.62 < 1 - \beta < .65$, two-sided tests) and a larger sample size would be needed for robust replication ($N = 126$). Detailed results of the power analyses are available in Supplementary Table 18.

4. Discussion

The present study investigated the roles of personality and a dopaminergic gene polymorphism in long-term fear extinction. Previously genotyped participants were recruited based on their dopaminergic *COMT* Val158Met polymorphism and performed a two-day differential fear conditioning paradigm with acquisition and extinction phases on Day 1 and a recall test on Day 2.

With regard to personality, three relevant findings emerged. First, although we assessed neuroticism/anxiety with five well established and highly reliable scales and assessed fear conditioning and extinction in three different conditioning phases and with five different behavioral, electrocortical and peripheral fear markers (which were all sensitive to fear conditioning across the entire sample), no direct correlation between any measures of conditioned or extinguished fear and neuroticism/anxiety could be detected. Only an exploratory analysis on the transfer from Day 1 extinction to Day 2 extinction recall in fear

bradycardia provided some tentative evidence for reduced extinction stability in emotionally unstable individuals. Second, agentic extraversion was also uncorrelated with any measures of Day 1 or Day 2 conditioned fear. Third, while agentic extraversion and neuroticism/anxiety were unrelated to conditioned and extinguished fear responses, dispositional fearfulness did predict conditioned fear during Day 1 acquisition as measured with fear bradycardia and unpleasantness ratings.

With regard to genetics, we found that *COMT* Val158Met predicted successful fear and extinction recall on Day 2 as measured with fear bradycardia and the LPP. At the beginning of the recall test, one day after conditioning and extinction, Val homozygotes but not Met carriers showed significantly enhanced LPP amplitudes and enhanced cardiac slowing to the non-extinguished vs. extinguished CS+. Meanwhile, Met homozygotes initially showed enhanced LPP amplitudes to both, previously extinguished and non-extinguished CS+. These results are consistent with previous rodent data, where Met homozygotes showed more freezing to an extinguished CS+ in the first four trials of an extinction recall test compared to Val homozygotes (Risbrough et al., 2014) and suggest an advantage for the Val allele in long-term fear extinction.

When comparing genotype groups, differences in fear and extinction recall were mainly qualified by (a) reduced non-extinguished fear responses (but comparably low extinguished fear responses) in Met carriers relative to Val homozygotes and (b) failed reduction in extinguished fear bradycardia in Val/Met compared to Val/Val carriers. Taken together, Val homozygotes showed the most adaptive response pattern, reflecting actual Day 1 CS-US contingencies in their responses while Met carriers showed patterns inconsistent with previous CS-US contingencies. Exploratory analyses revealed that failure of Met carriers to produce a reduced fear bradycardia to previously extinguished fear stimuli was particularly driven by those Met carriers who already failed to reduce fear during Day 1 extinction. As stated above, this Day-1-Day-2 transfer was also compromised in individuals with high neuroticism/anxiety such that emotionally instable individuals showed high Day-1-Day-2 fluctuations. While *COMT* Val158Met modulated long-term learning, there was no direct relation to within-session/short-term fear acquisition or extinction. This pattern of findings indicates that the *COMT* Val158Met polymorphism contributes to individual differences in long-term fear learning (Bellander et al., 2015).

As the COMT enzyme is primarily related to prefrontal dopamine degradation (e.g., Yavich et al., 2007), the present study supports the notion that prefrontal dopamine modulates *retention* of fear extinction rather than within-session extinction (Abraham et al., 2014). Consistent with other studies we found no *COMT* Val158Met effects for Day 1 extinction (Gruss et al., 2016; Norrholm et al., 2013; Raczka et al., 2011) but instead for Day 2 recall. One previous study in humans found stronger fear responses in Met homozygotes (Lonsdorf et al., 2009) during extinction 24 h after initial fear conditioning which was later interpreted as increased long-term fear retention (Lonsdorf & Kalisch, 2011). In line with the present result pattern, this supports a role of *COMT* Val158Met for memory retention rather than fear responding per se. As our results suggest generally more stable fear retention in Val/Val carriers rather than Met/Met carriers, follow-up studies may inform about possible determinants of the relationship between *COMT* Val158Met and long-term fear retention inherent to the experimental design. One advantage of the present study is the use of a fully crossed (i.e., 2 × 2) differential conditioning and differential extinction design, which allows to disentangle *COMT* Val158Met effects on long-term fear retention and long-term extinction retention. Taken together, the present results indicate that Val homozygotes show better fear *and* extinction retention than Met carriers in the LPP and HP, at least at a group level when individual differences in Day 1 extinction success are discarded.

Although retention effects might stem from differences in both consolidation and/or recall of extinction memory, prefrontal dopamine – and thereby COMT enzyme expression – might be particularly relevant for consolidation as indicated by studies on endogenous prefrontal dopamine release in rats after extinction training (Hugues, Garcia, & Léna, 2007) and the finding that L-DOPA administration after extinction training improves long-term fear extinction in humans (Haaker et al., 2013; Haaker, Lonsdorf, & Kalisch, 2015). Future studies using COMT inhibitors could test directly to which extent *COMT* Val158Met modulates extinction consolidation and recall, respectively (Farrell, Tunbridge, Braeutigam, & Harrison, 2012; Giakoumaki, Roussos, & Bitsios, 2008).

We suggest that *COMT* Val158Met influences long-term fear extinction via prefrontal dopamine, with relatively lower dopamine levels (i.e., Val/Val) promoting generally better consolidation across all subjects, independent of individual differences in Day 1 extinction success. Nevertheless, the underlying mechanisms might go beyond a linear and direct effect of prefrontal dopamine levels on long-term fear extinction success. First, relationships between prefrontal functioning and extracellular dopamine levels seem to be inversely U-shaped (Durstewitz & Seamans, 2008; Farrell et al., 2012; E.M. Mueller, Burgdorf, Chavanon, Schweiger, Hennig, et al., 2014a; E.M. Mueller, Makeig, Stemmler, Hennig, & Wacker, 2011). Beyond the PFC, the relevant neural network likely extends to the ventral striatum which is involved in fear extinction via prediction error coding (Abraham et al., 2014). Moreover, concurrent dopaminergic activation of striatum and amygdala may be necessary for long-term learning (LaLumiere & Nawar, 2005). *COMT* Val158Met affects phasic dopaminergic firing in the striatum in a more subtle way than in the PFC (Bilder et al., 2004) and likely interacts with other dopaminergic genotypes (Felten, Montag, Markett, Walter, & Reuter, 2011; Raczka et al., 2011). Interestingly, the agency facet of extraversion, which has been consistently related to individual differences in dopamine in general and to *COMT* Val158Met in specific (Depue & Collins, 1999; E. M. Mueller, Burgdorf, Chavanon, Schweiger, Wacker, et al., 2014b), was unrelated to both *COMT* Val158Met and Day 2 extinction recall in the present study. Although variations in agentic extraversion have been linked to differences in frontostriatal dopaminergic activity, different psychological processes (e.g., incentive motivation in agentic extraverts vs. extinction retention processes) may be mediated via different neuron populations or distinct frontostriatal connections (Bromberg-Martin, Matsumoto, & Hikosaka, 2010; Salamone & Correa, 2012). Future studies might combine neuroimaging with pharmacological challenges and assess more genotypes in order to disentangle these complex mechanisms.

It should also be noted that remarkable individual differences in extinguished fear bradycardia (i.e., CS+E vs. CS−E) during initial Day 2 recall test were observed, particularly within Met carriers. Exploratory analyses revealed that these individual differences were partly driven by the level of remaining fear at the end of the Day 1 fear extinction. The correlation of difference scores (CS+E vs. CS−E) at the end of Day 1 extinction and initial Day 2 recall (a) was higher in Met vs. Val/Val carriers and (b) decreased with higher levels of neuroticism/anxiety. The stronger Day-1-Day-2 relationship in Met carriers again suggests that prefrontal dopamine levels modulate long-term extinction of fear bradycardia. The impaired extinction retention in some Met carriers of the present study is consistent with increased freezing behavior to previously extinguished CS+ in Met/Met vs. Val/Val rodents (Risbrough et al., 2014). In the present study, this effect was most pronounced in individuals who previously showed poor within-session extinction.

With regard to neuroticism/anxiety we did not find any direct associations with conditioned fear responses, which converges with a series of previous studies (Fredrikson & Georgiades, 1992; Grillon et al., 2006; Joos et al., 2012; Lommen et al., 2010; Martínez et al., 2012; Otto et al., 2007; Pineles et al., 2009). Instead, fearfulness significantly predicted fear bradycardia during Day 1 acquisition. Meanwhile, the two measures were uncorrelated, which is in line with weak or non-existing relationships of dispositional fear and anxiety in previous

studies (Depue & Lenzenweger, 2005; Sylvers et al., 2011). Furthermore, only fearfulness was associated with the *COMT* Val158Met genotype. It has previously been argued that high neuroticism/anxiety levels promote risk assessment in ambiguous situations rather than stronger defensive reactions to clearly threatening stimuli per se (e.g., Blanchard et al., 2001; Perkins & Corr, 2006). As mentioned earlier, in classical fear conditioning paradigms, implications of conditioned threat stimuli are well predictable and rather unambiguous. Moreover, *fear* reactions can be understood as short-lived and transient responses, beginning after detection of specific threat cues (i.e., CS+) and ending shortly after threat (i.e., after time window of potential US). Meanwhile, states of *anxiety* are defined as prolonged periods of risk assessment within ambiguous or uncertain contexts rather than in response to specific threat cues (Depue & Lenzenweger, 2005). Here, we stress the notion that individual differences in fearfulness (i.e., the disposition to strongly react to predictable, imminent harm) are better suited to predict fear acquisition in classical conditioning paradigms compared to neuroticism/anxiety. This is an important implication given that to date most fear conditioning studies use neuroticism/anxiety-related measures of individual differences (such as the STAI) rather than measures of fearfulness (Lonsdorf & Merz, 2017).

On the other hand, we showed for the first time, that neuroticism/anxiety may be linked to the Day-1-Day-2 stability of extinguished fear rather than the absolute amount of conditioned or extinguished fear at a single point in time. It is tempting to assume that emotional stability might be an important predictor for stability of extinction memory or even vice versa: the stability of safety learning may be important for emotional stability. This assumption is supported by reported associations of anxiety (but not fear) with volume and activity in the hippocampus (e.g., Kalisch et al., 2006; Montag, Reuter, Jurkiewicz, Markett, & Panksepp, 2013; Satpute, Mumford, Naliboff, & Poldrack, 2012), a core structure in various memory processes (e.g., Izquierdo & Medina, 1997; Maren & Holt, 2000). However, as these observations were based on exploratory analyses, future studies should replicate these results on neuroticism/anxiety, *COMT* Val158Met, and the specific associations of fear extinction success and fear extinction recall. Our differential result pattern of (a) *COMT*-related differences in fearfulness, (b) fearfulness predicting short-term fear acquisition and (c) neuroticism/anxiety predicting long-term extinction stability highlights the importance of measuring sufficiently precise behavioral phenotypes in order to find associations with responses in the laboratory and with genotypes (Wacker et al., 2012).

The present results are potentially relevant for understanding anxiety disorders and their treatment. Both *COMT* Val158Met (Lonsdorf & Kalisch, 2011; Montag, Jurkiewicz, & Reuter, 2012) and neuroticism/anxiety (Clark et al., 1994; Mineka, Watson, & Clark, 1998; Weinstock & Whisman, 2006) have been linked to the prevalence of anxiety disorders. Given the present effects exclusive for *long-term* fear extinction, *COMT* Val158Met and neuroticism/anxiety might primarily relate to maintenance and treatment resistance (Lonsdorf et al., 2010) which should be investigated in future studies. Moreover, future studies investigating mechanisms of anxiety disorders in subclinical samples may include fearfulness as a potential predictor for the acquisition of dysfunctional fear reactions. The contributions of neuroticism/anxiety and fearfulness vary between different anxiety disorders and fearfulness might be especially relevant for the *development* of phobias and panic disorder (Kampman, Viikki, & Leinonen, 2017; Lang et al., 2000; McNaughton & Corr, 2004), whereas neuroticism/anxiety may be more relevant for maintenance and treatment success.

Some limitations should be addressed. First, we used a high number of trials to achieve a sufficient signal-to-noise ratio in ERP measures. This required the use of noise burst USs rather than electric shock US (Sperl et al., 2016), which are more commonly used in behavioral and neuroimaging fear conditioning research. This methodological difference should be kept in mind, when comparing the present results to other studies. Nevertheless, we evoked robust CRs on Day 1 and Day 2. Second, relevant subcortical structures like the amygdala (LeDoux, 2000) cannot be assessed with EEG. Future fMRI studies should therefore address the interplay of *COMT* Val158Met, neuroticism/anxiety and amygdala in extinction retention. Third, because sex and menstrual cycle influence fear learning (Lebron-Milad et al., 2012; Merz et al., 2012) and dopamine (Risbrough et al., 2014) this initial study aimed to control for these factors by only testing males. Future studies will have to investigate interactions between sex and *COMT* Val158Met in human long-term fear extinction to probe the generalizability of the present findings to females. Fourth, there was an unexpected significant Genotype × Extinction interaction in fear bradycardia during Day 1 acquisition. This effect likely was due to a type I error, given that the factor Extinction had not been manipulated yet. Moreover, interpretation of Day 2 results is not affected as the relevant three-way interaction (Genotype × Contingency × Extinction) was not present on Day 1. Finally, it should be kept in mind that we assessed several independent (*COMT* Val158Met, fearfulness, neuroticism/anxiety, agentic extraversion) and dependent (LPP, fear bradycardia, SCR, affective self-reports) variables and reported different analytic approaches (averaging across all trials vs. only assessing the first or last ten trials). While this obviously resulted in a high number of tests which increases the likelihood of false positive results, we believe that such an approach is necessary, given that conditioned fear is a dynamic and multilevel phenomenon with potential relevance for various personality domains. It should further be noted that this study and the collected sample were a priori designed to primarily test very specific effects which turned out to be significant, namely the influence of *COMT* Val158Met on Day 2 cortical and cardiac fear responses. Nevertheless, future replications will be necessary. Here, power analyses suggested that relationships between personality and fear bradycardia as well as between *COMT* Val158Met and Day 2 fear bradycardia had sufficient statistical power in the present study and could be replicated using comparable or smaller sample sizes. Meanwhile, studies trying to replicate *COMT* Val158Met effects on Day 2 LPP and Day-1-Day-2 stability in fear bradycardia should have more power.

For the first time, we provide evidence for *COMT* Val158Met modulation of *long-term* fear extinction in humans, evident both in cortical (LPP) and autonomic (fear bradycardia) components of the conditioned fear response. We further found that the initial acquisition of fear is related to dispositional fearfulness and provide tentative evidence that the stability of extinction memories may be relevant for neuroticism/anxiety. The present findings are of potential relevance for the understanding of dispositional fearfulness and neuroticism/anxiety, as well as development and treatment of anxiety disorders.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.nlm.2018.06.001.

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4.6 Manuscript 6:
Fear Extinction Recall Modulates Human Frontomedial Theta and Amygdala Activity

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Scientific Recognition:

I received two young scientists awards for this publication: The **Brain Products Young Scientist Award for a Distinguished Contribution in EEG Research**, which was awarded by the **German Society for Basic and Applied Psychophysiology** (Deutsche Gesellschaft für Psychophysiologie und ihre Anwendung; DGPA) and **Brain Products** (Munich); and the **WASAD Young Researcher Award**, which was awarded by the **World Association for Stress Related and Anxiety Disorders** (WASAD).

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Original Article

ORIGINAL ARTICLE

Fear Extinction Recall Modulates Human Frontomedial Theta and Amygdala Activity

Matthias F. J. Sperl[1,2,3], Christian Panitz[1,2], Isabelle M. Rosso[3], Daniel G. Dillon[3], Poornima Kumar[3], Andrea Hermann[2], Alexis E. Whitton[3], Christiane Hermann[2], Diego A. Pizzagalli[3] and Erik M. Mueller[1,2]

[1]Department of Psychology, Personality Psychology and Assessment, University of Marburg, 35032 Marburg, Germany, [2]Department of Psychology, Clinical Psychology and Psychotherapy, University of Giessen, 35394 Giessen, Germany and [3]Department of Psychiatry, Center for Depression, Anxiety and Stress Research, McLean Hospital, Harvard Medical School, Belmont, MA 02478, USA

Address correspondence to Matthias F.J. Sperl, Department of Psychology, Personality Psychology and Assessment, University of Marburg, Gutenbergstr. 18, 35032 Marburg, Germany. Email: matthias.sperl@staff.uni-marburg.de

Abstract

Human functional magnetic resonance imaging (fMRI) and electroencephalography (EEG) studies, as well as animal studies, indicate that the amygdala and frontomedial brain regions are critically involved in conditioned fear and that frontomedial oscillations in the theta range (4–8 Hz) may support communication between these brain regions. However, few studies have used a multimodal approach to probe interactions among these key regions in humans. Here, our goal was to bridge the gap between prior human fMRI, EEG, and animal findings. Using simultaneous EEG–fMRI recordings 24 h after fear conditioning and extinction, conditioned stimuli presented (CS+E, CS−E) and not presented during extinction (CS+N, CS−N) were compared to identify effects specific to extinction versus fear recall. Differential (CS+ vs. CS−) electrodermal, frontomedial theta (EEG) and amygdala responses (fMRI) were reduced for extinguished versus nonextinguished stimuli. Importantly, effects on theta power covaried with effects on amygdala activation. Fear and extinction recall as indicated by theta explained 60% of the variance for the analogous effect in the right amygdala. Our findings show for the first time the interplay of amygdala and frontomedial theta activity during fear and extinction recall in humans and provide insight into neural circuits consistently linked with top-down amygdala modulation in rodents.

Key words: fear conditioning, fear extinction, frontal-midline theta, simultaneous EEG–fMRI, threat processing

Introduction

Elucidating brain mechanisms of conditioned and extinguished fear recall is crucial for understanding pathological processes underlying anxiety disorders and for developing interventions to enhance extinction learning (Bowers and Ressler 2015). Anatomically, human fear expression is associated with increased activation in the amygdala (Kim and Jung 2006; LeDoux 2014; but see Fullana et al. 2016), insula (Kim and Jung 2006; Fullana et al. 2016), and anterior midcingulate cortex (AMC) (Milad et al. 2007a; Fullana et al. 2016), whereas recall of extinguished fear is commonly linked to increased ventromedial prefrontal cortex (vmPFC) activation (Kalisch et al. 2006; Milad et al. 2007b; Milad and Quirk 2012; Hermann et al. 2016) and decreased amygdala activation (Phelps et al. 2004; Hermann et al. 2016). The amygdala is thought to mediate fear learning and fear expression (LeDoux 2014; Hermans et al. 2017). It serves as a hub for fear-related processes (Milad and Quirk 2012; Kim and Cho 2017), receiving input from prefrontal

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regions involved in fear expression and regulation (Hartley and Phelps 2009; Pitman et al. 2012). Importantly, the AMC has excitatory projections to the amygdala during fear recall (Gilmartin et al. 2014), which regulate physiological fear responses (Hartley and Phelps 2009; Panitz et al. 2015). Conversely, inhibition of the fear response during extinction recall is mediated by projections from the vmPFC to intercalated cells in the amygdala (Quirk and Mueller 2008; Pitman et al. 2012), presumably modulated by hippocampal activation (Milad and Quirk 2012; Merz et al. 2014).

Studies investigating fear extinction in rodents have identified homologous prefrontal brain regions. Specifically, stimulation of the rodent prelimbic cortex (PL), which is considered the homolog of the human AMC (Milad and Quirk 2012), increases fear expression (Vidal-Gonzalez et al. 2006). Similarly, inactivation of the infralimbic cortex (IL), a homologous region to the human vmPFC (Milad and Quirk 2012), impairs fear extinction (Sierra-Mercado et al. 2011; Lingawi et al. 2016). Although there is evidence from rodent single-cell recording studies that the amygdala is crucial for triggering fear responses (Repa et al. 2001), the duration of CS evoked amygdala responses is very short (Quirk et al. 1995; Goosens and Maren 2004). Conversely, PL theta (i.e., 4–8 Hz) oscillations are assumed to be relevant for initiating more sustained fear processing at neural and behavioral levels (Burgos-Robles et al. 2009; Pitman et al. 2012). Specifically, rodent PL neurons show a sustained response to previously fear-conditioned and nonextinguished stimuli by a change in their firing rate from 2 Hz to the theta range (i.e., ~4–8 Hz; Burgos-Robles et al. 2009), and theta synchrony may be crucial for amygdala-AMC connectivity (Gilmartin et al. 2014). Importantly, the PL may receive information about CS salience from the amygdala (Gilmartin et al. 2014; Senn et al. 2014), while projections from the PL to the amygdala may provide information regarding the predictive value of the CS (Courtin et al. 2014; Gilmartin et al. 2014).

Converging with these animal studies, a recent human 64-channel EEG study (Mueller et al. 2014b) showed that healthy subjects displayed enhanced theta oscillations (4–8 Hz) at frontomedial EEG electrodes during the presentation of previously fear-conditioned and nonextinguished stimuli, which were source-localized to the AMC (Mueller et al. 2014b). Consistent with a key role in fear expression and extinction, frontal-midline theta has also been consistently linked to state and trait anxiety in humans (Mitchell et al. 2008; Mueller et al. 2014a; Cavanagh and Shackman 2015) and is modulated by anxiolytic drugs (Mitchell et al. 2008). Importantly, brain oscillations not only relate to threat processing, but also can be conceptualized as reflecting neural mechanisms of cognitive processes (Lopes da Silva 2013). Synchronous oscillations are crucially involved in linking brain areas within functional networks (Klimesch 1996; Bastiaansen, Mazaheri, Jesen 2012). Theta oscillations are of particular relevance for modulating and gating information transfer among specific neuronal populations (Mizuseki et al. 2009; Lopes da Silva 2013), including communication between prefrontal brain areas and the amygdala (Gilmartin et al. 2014). Notably, in mice, altered theta synchronization in the amygdala–prefrontal cortex network has been associated with fear extinction recall (Narayanan et al. 2011).

While animal studies have significantly helped to develop plausible neural models of fear learning, the limited temporal and spatial resolution of functional magnetic resonance imaging (fMRI) and electroencephalography (EEG), respectively, have limited the generalization of insights from animal single-cell recording studies to humans. Taken together, electrophysiological findings suggest that frontomedial theta oscillations are essential for anxiety and fear-related processes not only in animals (Likhtik and Gordon 2014), but also in humans (Mueller et al. 2014a, 2014b; Cavanagh and Shackman 2015). Conversely, fMRI has been widely used to study fear conditioning and extinction in humans (Milad and Quirk 2012), and has consistently highlighted the amygdala as a hub region for fear processing (Phelps and LeDoux 2005; Janak and Tye 2015). However, because these findings emerge from different imaging modalities, it remains unclear how they can be integrated and how amygdala processes and theta oscillations are functionally connected in humans. In particular, the integration of models for amygdala activation with frontomedial theta oscillations cannot be assessed with fMRI or EEG in isolation. The aim of the present study is to bridge the gap between prior animal studies, human EEG, and human fMRI findings by recording EEG and fMRI simultaneously.

To address this question, we used an established 2-day fear conditioning and extinction paradigm (Fig. 1) (Mueller et al. 2014b). During fear acquisition, 2 conditioned stimuli (CS+) were repeatedly paired with an aversive unconditioned stimulus (US), while 2 additional conditioned stimuli (CS–) were never followed by a US. In the subsequent extinction phase, 1 of the 2 CS+ ("CS+E") and one CS– ("CS–E") were presented without the US, and thus responses to those stimuli were extinguished. The other CS+ ("CS+N") and CS– ("CS–N") were not presented, thus leaving learned responses to those stimuli fully intact. During a recall test approximately 24 h later, EEG and fMRI were recorded simultaneously. To identify effects specific to extinction versus fear recall, differential hemodynamic and electrophysiological responses to extinguished (CS+E vs. CS–E) and nonextinguished conditioned stimuli (CS+N vs. CS–N) were compared. Our data revealed the expected interplay of amygdala activation and frontomedial theta oscillations, thus extending key insights from animal research into the human realm. Theta activity appears to play a dominant role in communication between the amygdala and the prefrontal cortex during human fear and extinction recall (FER).

Materials and Methods

Subjects

A total of 21 healthy students at Justus Liebig University Giessen were recruited for this study. Three subjects were excluded from the analysis due to complete absence of explicit CS–US contingency awareness after acquisition (defined as higher awareness ratings for CS– than CS+), resulting in a final sample of 18 right-handed and nonsmoking subjects (mean age = 22.72 years, standard deviation [SD] = 3.34 years, range: 19–29 years; 50% females; see also Supplementary Material H). All subjects participated either for partial fulfillment of course credit or were reimbursed with 10 €/h, and gave written informed consent to participate. As there is evidence for an influence of menstrual cycle phase on fear conditioning and extinction (Hwang et al. 2015), only female participants who took oral contraceptives on a regular basis were recruited. Moreover, they were tested during their pill intake phase in order to reduce variance related to fluctuations of gonadal hormones. Exclusion criteria were a history of mental (assessed by the short version of the Diagnostic Interview for Mental Disorders, Mini-DIPS; Margraf 1994), neurological, or cardiovascular disorders,

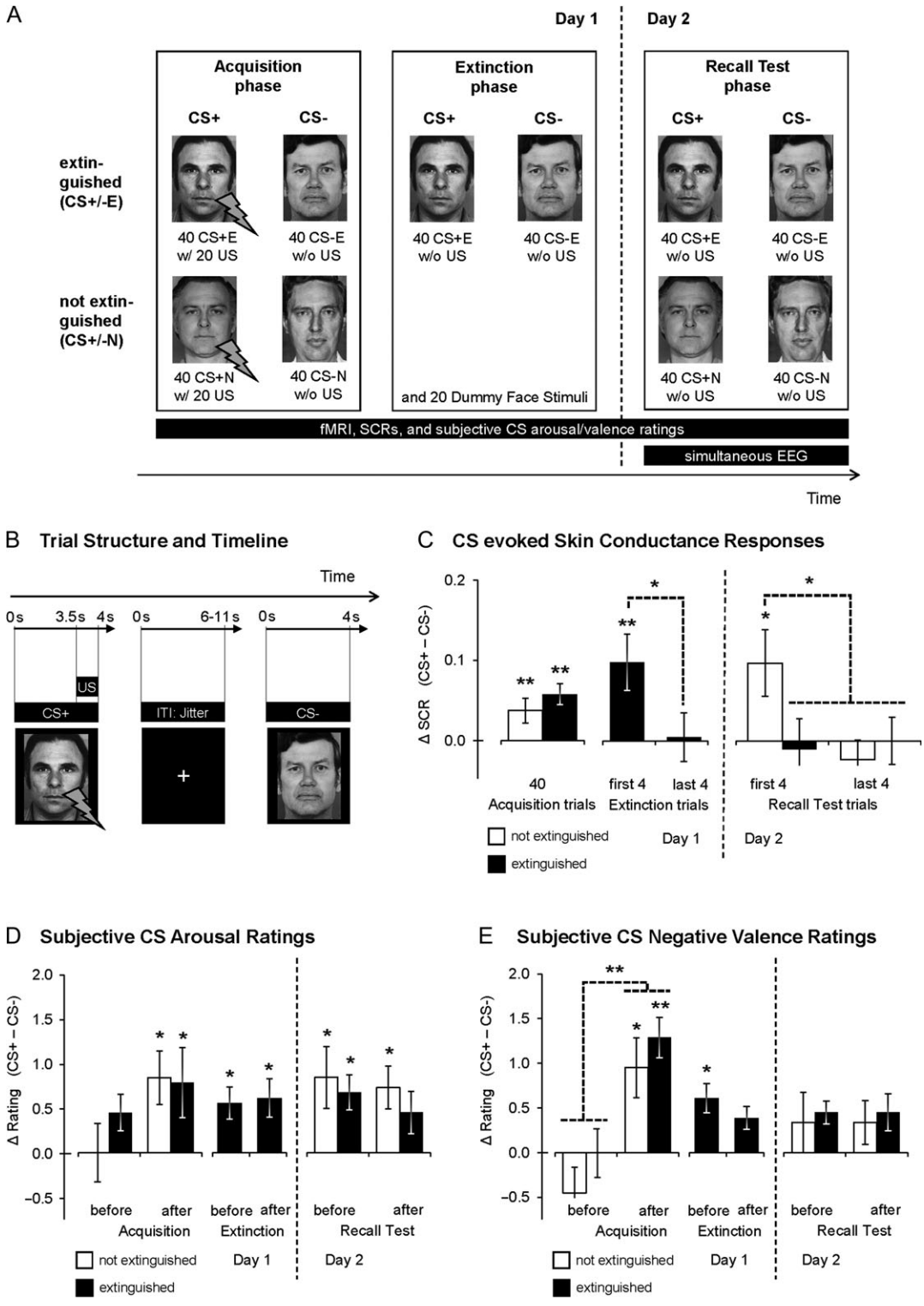

Figure 1. Schematic depiction of the experimental paradigm used in the present study. (*A*) Number and stimuli types presented during the 3 experimental phases. The central hypotheses of the current study focused on the Day 2 recall test, during which EEG and fMRI were recorded simultaneously. CS+E/CS–E, CSs presented during extinction phase; CS+N/CS–N, CSs not presented during extinction phase. CS+ were reinforced with an aversive US ("w/", contingency of 50%) during acquisition phase, while CS– were never paired with a US ("w/o"). (*B*) Trial structure and timeline for a single CS trial. All CSs were shown for 4 s. During the acquisition phase, a 500-ms electric shock US coterminated with 50% of all CS+ trials, starting 3.5 s after CS onset. (*C*) Normalized CS evoked differential (CS+ – CS–) SCRs, (*D*) subjective CS arousal ratings, and (*E*) subjective CS negative valence ratings ($M \pm$ within-subject standard error of the mean (SEM), O'Brien and Cousineau 2014) for extinguished and nonextinguished stimuli during all experimental stages. $^{*}P \leq 0.05$, $^{**}P \leq 0.01$ (one-sided, CS+ > CS–).

or a report of MRI exclusion criteria. Furthermore, subjects were excluded if they reported using illegal drugs or prescription drugs that affect the central nervous system. All subjects had normal or corrected-to-normal vision and were asked to refrain from alcoholic or caffeinated drinks, heavy meals, and strenuous exercise prior to the experiment. The study protocol was approved by the local ethics committee of the Faculty of Psychology and Sports Science at Justus Liebig University Giessen.

Experimental Paradigm

A 2-day fear conditioning and extinction paradigm (Mueller et al. 2014b) was adapted for simultaneous EEG–fMRI recordings (Fig. 1). During acquisition (Day 1), 2 CS+ (CS+E, CS+N) and 2 CS– (CS–E, CS–N) were presented 40 times each in random order, while both CS+ coterminated with an aversive US in 50% of the trials. All CSs were shown 4 times prior to acquisition without any US pairings to familiarize participants with the stimuli. Approximately 20 min after acquisition, subjects completed an extinction phase, during which only 1 of the 2 CS+ (i.e., extinguished CS+, CS+E) and 1 of the 2 CS– (i.e., CS–E) were presented 40 times each in random order. The other 2 CSs (i.e., nonextinguished CSs, CS+N and CS–N) and the US were not presented during extinction. In order to maintain some variability of stimuli shown during extinction, a novel face ("Dummy Stimulus") was presented 20 times. Approximately 24 h later, all extinguished and nonextinguished stimuli were shown 40 times each in random order without any US presentation. Recall of extinguished fear can be distinguished from recall of conditioned fear by comparing extinguished (CS+/–E) and nonextinguished (CS+/–N) stimuli.

Conditioned and Unconditioned Stimuli

Four different black-and-white pictures of male faces with a neutral expression (Ekman and Friesen 1976) constituted the CSs. The assignment of face stimuli to CS+E, CS+N, CS–E, and CS–N was permutated in a counterbalanced fashion. We confirmed that after the exclusion of 3 contingency unaware subjects (see Subjects) reasonable counterbalancing was still achieved (Supplementary Material A). Specifically, there was no significant association between any CS type (e.g., CS+E) and assignment of particular face stimuli, $x^2(3) = 1.11$, exact $P = 0.859$. All faces were presented for 4 s with a jittered intertrial interval (defined as CS offset to CS onset) of 6–11 s. During the intertrial interval, a white fixation cross was shown on a black background. Visual stimuli were presented on an MR-compatible 32-in visual stimulation system (NordicNeuroLab, Bergen, Norway), while subjects were able to look at the screen by a mirror that was mounted to the head coil (visual angle = 28°). An eye camera (ViewPoint PC-60, Arrington Research, Scottsdale, AZ, USA) was also placed at the head coil in order to check whether subjects had their eyes open and watched the stimuli.

The US consisted of a 500-ms multipulse (1-ms pulses, 50 Hz) electrical stimulation which was delivered from a transcutaneous current stimulator (E13-22, Coulbourn, Allentown, PA, USA) using 2 custom-made steel disk electrodes attached to the middle of the left lower leg (surface size: 1.8 mm^2). During a work-up procedure, the intensity of the shocks ($M = 1.76$ mA, $SD = 0.92$ mA) was set individually to a level which was subjectively perceived as "difficult to bear, but acceptable." Additionally, participants had to rate negative valence of the US higher than 6 on an 11-point Likert scale (0 = not unpleasant at all, 10 = extremely unpleasant) at least 3 times in a row. As the paradigm consists of many trials, which are necessary to ensure an adequate signal-to-noise ratio for EEG analyses, habituation to the US is a potential issue, when conventional shock intensities are used (Sperl et al. 2016). We therefore used a work-up procedure that leads to a slightly higher shock intensity compared with previous peripheral physiological or fMRI studies on fear conditioning (e.g., compared with Hermann et al. 2016). Shock electrodes were attached during all experimental phases.

Subjective CS Ratings

Prior to and after each experimental stage, subjects were asked to rate perceived arousal (1 = not arousing; 5 = very arousing) and valence (1 = very pleasant; 5 = very unpleasant) of each CS on a 5-point Likert scale. For the extinction phase, ratings were restricted to CS+E and CS–E. During acquisition and Day 2 recall phases, additional ratings were requested in the middle of the experimental stages. In addition, subjective awareness of the CS–US contingency was assessed on a 4-point Likert scale (0 = CS was never followed by US; 3 = CS was always followed by US) after acquisition.

In order to evaluate conditioning and extinction on subjective ratings, three-way repeated-measures analyses of variance (ANOVAs) with "Contingency" (CS+ vs. CS–), "Extinction Status" (E vs. N) and "Time" (prior to vs. after acquisition, extinction, or recall phase, respectively) were carried out for each experimental phase. The factor Time was included as we expected an increase of differential ratings (CS+E, CS+N vs. CS–E, CS–N) during acquisition followed by a decrease during extinction. Importantly, FER on Day 2 can be assessed by comparing differential ratings for nonextinguished versus extinguished CSs prior to the recall test.

SCR Data Acquisition and Analyses

Skin conductance was recorded using an additional channel (GSR-MR sensor) of the BrainAmp-MR EEG system (Brain Products, Munich, Germany). Two Ag/AgCl electrodes of a 6-mm diameter filled with isotonic (0.5% NaCl) electrolyte medium were placed on the hypothenar eminence of the left hand. Data were low-pass filtered online (Day 1: 250 Hz, sampling rate 1 kHz; Day 2 during simultaneous EEG: 1 kHz, sampling rate 5 kHz), and afterwards a 0.5 Hz low-pass filter was applied offline. After manually checking for artifacts, for each CS trial a skin conductance response (SCR) score was calculated (Milad et al. 2007b) by subtracting the peak response within 5 s after CS onset from a 1 s pre-CS baseline. This approach, that is, calculating the cumulative maximum conductance change after CS onset for quantification of SCRs (rather than distinguishing between early and late intervals) has been recommended by Pineles, Orr, and Orr (2009) for CS–US intervals as in the present study and is consistent with many human fear conditioning studies (Lonsdorf et al. 2017). Following established procedures (Lykken and Venables 1971), individual SCRs were normalized by dividing the raw SCR value of each CS by an individual's maximum SCR value across all CS (separately for experimental phases). Afterwards, SCR scores were averaged across trials for each CS type. During fear acquisition, successful conditioning is reflected by higher SCRs for unpaired CS+ (CS+E, CS+N) compared with CS– (CS–E, CS–N), which was tested using a two-way repeated-measures ANOVA with the factors Contingency (CS+ vs. CS–) and Later Extinction Status (E vs. N). We expected a decline of this conditioned response (CS+E vs. CS–E) from early (first 4 trials) to late (last 4 trials) extinction learning

(Milad et al. 2013, Contingency × Time ANOVA). Successful FER on Day 2 can be demonstrated by higher SCRs for CS+N compared with CS−N, but not for CS+E compared with CS−E. Due to a quick habituation of fear-conditioned SCRs (Lonsdorf et al. 2017), we expected this effect during the first 4 recall trials (as in prior studies; Milad et al. 2007b; Hermann et al. 2016), but not toward the end of the recall phase. Similar to the analyses on affective ratings, we computed a Contingency × Extinction Status × Time (first vs. last 4 recall trials) ANOVA. To explicitly test for a differential habituation of fear-conditioned and extinguished SCRs (i.e., fear recall leading to an elevated SCR response to the CS+N in the first 4 trials as compared with all other stimuli and as compared with the last 4 trials), we specified the transformation coefficients matrix for the following customized hypothesis test: [CS+N first 4 trials (contrast coefficient = +7)] vs. [CS+E first (−1), CS+E last (−1), CS+N last (−1), CS−E first (−1), CS−E last (−1), CS−N first (−1), CS−N last (−1) 4 trials].

fMRI Data Acquisition and Analyses

Functional and structural data were acquired using a Siemens MRI Scanner MAGNETOM Prisma (3.0 T, Siemens Healthineers, Erlangen, Germany) with an XR 80/200 gradient coil and a Head/Neck 64-channel coil. For functional images, T2*-weighted gradient echo-planar imaging sequences (Siemens WIP883A, based on ep2d_bold) with 40 slices covering the whole brain were applied (slice thickness: 3 mm, interslice gap: 0.75 mm; descending slice procedure; TR = 2500 ms; TE = 30 ms; flip angle = 75°; field of view: 192 × 192 mm^2; voxel size: 3 × 3 × 3 mm^3; GRAPPA: acceleration factor 2). For the acquisition and recall phases, 841 volumes were collected, while 507 volumes were acquired during extinction. In order to minimize susceptibility artifacts in prefrontal brain areas, orientation of axial slices was set with autoalign (Head-Brain) and an additional angle of −30° transversal to coronal. For the normalization procedure, 176 T1-weighted structural images (MPRAGE, slice thickness: 0.94 mm; TR = 1580 ms; TE = 2.3 ms; field of view: 240 × 240 mm^2; voxel size: 0.94 × 0.94 × 0.94 mm^3; GRAPPA: acceleration factor 3) were acquired in sagittal orientation. Moreover, a gradient echo field map was collected for unwarping of B_0 distortions.

All analyses of fMRI data were performed in SPM12 (Wellcome Department of Cognitive Neurology, London, UK), implemented in MATLAB 8.6 (MathWorks, Natick, MA, USA). Each experimental session was analyzed separately. Preprocessing of fMRI data included unwarping and realignment, slice time correction, co-registration to the structural image of each subject, segmentation into different tissue types, normalization ("unified model" implemented in SPM12 which includes linear and nonlinear transformations) to the standard space of the Montreal Neurological Institute (MNI) brain with a voxel size of 2 × 2 × 2 mm^3, and spatial smoothing with an isotropic 3D Gaussian kernel (FWHM: 4 mm). Furthermore, outliers in the temporal scan-to-scan difference series were identified using Artifact Detection Toolbox (ART; McGovern Institute for Brain Research, Cambridge, MA, USA). Extreme volumes with regard to global signal intensity (>3 SD of average signal intensity across scans) and translational movement (>0.5 mm) were modeled as outliers in the first-level analysis. In addition, head motion parameters (3 translation parameters, 3 rotation parameters, 1 composite motion parameter which contains the maximum scan-to-scan movement) were included as first-level regressors.

For the acquisition phase, the first-level general linear model (GLM) contained the following 3 task-related regressors: CS+ (CS+E and CS+N combined), CS− (CS−E and CS−N combined), and US. To confirm that neural responses did not differ between to-be extinguished and to-be nonextinguished stimuli during acquisition, we constructed an additional first-level GLM which contained separate regressors for to-be extinguished and to-be nonextinguished CS+/CS−. As the analysis on CS+ was restricted to unreinforced stimuli (not paired with US), this regressor was split into 2 regressors (paired CS+ and unpaired CS+). For the extinction phase, CS+E, CS−E, and Dummy Stimulus were included as regressors. For Day 2 recall, the first-level model consisted of CS+E, CS+N, CS−E, and CS−N. The ratings of CSs in the middle of the acquisition and recall phases were modeled as additional regressors, while volumes collected during the ratings at the beginning and end of each phase were discarded. All previously described regressors were modeled by a block function with the length of the events which was convolved with the hemodynamic response function in the GLM of the first-level analysis. In order to remove slow signal drifts, a high-pass filter with a time constant of 128 s was applied. For the acquisition and extinction stages, contrasts for conditioned responses (CS+ vs. CS−) were computed for each subject and tested in one-sample *t*-tests during the second-level random effects group analysis (i.e., *t*-tests for previously specified first-level contrasts > 0). For evaluating FER on Day 2, the contrast [(CS+N − CS−N) versus (CS+E − CS−E)] was calculated to compare differential fear responses for nonextinguished (CS+N − CS−N) and extinguished stimuli (CS+E − CS−E).

For all contrasts, both region of interest (ROI) analyses and exploratory whole brain analyses were performed. ROIs contained main structures that have been consistently implicated in fear and extinction (Milad and Quirk 2012; Hermann et al. 2016): amygdala, AMC, hippocampus, insula, and vmPFC. The masks for AMC and vmPFC were created in the MARINA software package (Walter et al. 2003) according to the parcellation of Tzourio-Mazoyer et al. (2002), and were identical to the ones used in previous studies (Hermann et al. 2009; Pejic et al. 2013; Hermann, Keck, Stark 2014). The AMC mask consists of the bilateral cingulate and paracingulate gyri and ranges from *y* = 32 to *y* = −18 (MNI coordinates) with regard to the AC-PC line (Supplementary Material B, see Supplementary Fig. S2A). This mask includes the 2 peak coordinates reported in a recently published meta-analysis on fear conditioning (Fullana et al. 2016). The vmPFC mask consists of the bilateral medial orbital area of the frontal cortex and the gyrus rectus (Supplementary Material B, see Supplementary Fig. S2B), including the 2 peak voxels identified by a meta-analysis on fear extinction (Diekhof et al. 2011). All other masks were maximum probability masks taken from the Harvard-Oxford Cortical and Subcortical Structural Atlases (Harvard Center for Morphometric Analyses, Charlestown, MA, USA) with the probability threshold at 0.50. For exploratory whole brain analyses, a significance threshold of $P \leq 0.05$ on voxel level (family-wise error [FWE] correction for multiple comparisons) with a minimal cluster size (*k*) of 10 voxels was used. All ROI analyses were computed using the small volume correction option of SPM12, while the significance threshold was set to $P \leq 0.05$ on voxel level (FWE-correction). With the exception of AMC and vmPFC masks, all ROIs were tested separately for the left and right hemisphere (Merz et al. 2014).

Analyses of BOLD responses were collapsed across all trials of each experimental phase. Previous fMRI studies of human fear conditioning accounted for a rapid decrease of CS evoked BOLD modulations over time, which is of particular relevance for recall tests without continuing US presentations (Büchel

et al. 1998; Armony and Dolan 2001; Milad et al. 2007b, 2013; Hermann et al. 2016). To increase comparability with other fMRI studies, we accounted for a decrease of BOLD activation over time for the analysis on Day 2 recall, that is, the experimental phase of critical relevance for our hypotheses. Specifically, there is evidence that habituation of amygdala activation can be best characterized by an exponentially decaying function (Büchel et al. 1998; Büchel et al. 1999; Armony and Dolan 2001). Furthermore, habituation of CS evoked SCRs during fear recall (Sperl et al. 2016; Lonsdorf et al. 2017) is correlated with amygdala habituation (Büchel et al. 1998; Phelps et al. 2004; Knight et al. 2005). Consequently, when considering the exponentially decaying function $y = a \cdot e^{-bx}$ we estimated parameters a and b for showing the best fit to the trial-wise habituation of group and CS condition averaged SCRs (Curve Fitting Toolbox 3.5.2, implemented in MATLAB 8.6; MathWorks, Natick, MA, USA). Due to considerable variance of mean SCRs during the second half of the recall test, curve fitting was limited to SCRs of the first half, resulting in $a = 0.28$ and $b = 0.35$ (goodness of fit: $R^2 = 0.62$). Afterwards, for each CS type, an additional regressor was added in the first-level model as parametric modulator which was multiplied with the previously fit function for all recall trials.

To enhance comparability with studies of Milad and colleagues (Milad et al. 2007b, 2009; Milad et al. 2013; Hermann et al. 2016) and to further evaluate the validity of our findings, we performed an additional analysis for Day 2 recall which was restricted to the first 4 trials of each CS type. Therefore, instead of applying an exponential modulation, CS regressors of the first-level GLM were split into 10 regressors of 4 trials each. Our main findings on amygdala activation could be confirmed with both strategies. Corresponding to previous studies on fear/extinction recall (Milad et al. 2009), no significant brain correlates could be found if we did not use any of these strategies to account for habituation of BOLD responses over time.

Finally, for illustration purposes and to perform post hoc control analyses, we extracted contrast estimates using MarsBaR Toolbox (Brett et al. 2002). Contrast estimates represent mean values for an activated cluster of voxels with $P \leq 0.005$ (uncorrected) surrounding FWE-corrected activation peaks.

EEG Data Acquisition and Analyses

During Day 2 recall, EEG was recorded simultaneously inside the MRI scanner (BrainAmp-MR, Brain Products, Munich, Germany), using 31 sintered Ag/AgCl ring electrodes attached to the EEG cap (BrainCap-MR 32 Channels, Easycap, Herrsching, Germany). During recording, an additional electrode at FCz served as reference and an electrode at AFz was used as ground electrode. Electrode impedance was kept below 5 kΩ prior to recording. One remaining channel of the EEG system was used to record the electrocardiogram (ECG), which was used for subtracting heartbeat artifacts during the EEG analysis. In order to prevent pump-induced subject movements, the helium-pump of the MR system was switched off during simultaneous EEG–fMRI. Furthermore, the clock of the EEG system and of the MRI gradient system were synchronized (SyncBox, Brain Products, Munich, Germany) to enhance the quality of MRI artifact subtraction procedures for EEG data and to reduce timing-related errors. The sampling rate was 5 kHz, which is required for artifact reduction procedures. EEG and ECG were band-pass filtered (0.016–250 Hz) online.

EEG preprocessing was performed in BrainVision Analyzer 2.0.2 (Brain Products, Munich, Germany). Corrections for MR gradient and cardioballistic artifacts were applied to EEG data according to adapted versions (Sammer et al. 2005) of the algorithms described by Allen and colleagues (Allen et al. 1998, 2000). A scanner artifact template was created, containing only little EEG contribution, by averaging all EEG segments which interfered with fMRI scanning. The volume-marker of the MR scanner was used to detect scanner artifacts and the segment length was one TR. This correction template was subtracted from each EEG segment. In order to remove residual frequencies without physiological origin, data were low-pass filtered (cutoff at 40 Hz). Cardioballistic artifacts were reduced in a second step. Similar to the reduction of gradient artifacts, an average pulse curve (derived from the 12–20 Hz notch filtered ECG data) was subtracted from the EEG. The correction method accounted for the time delay between the heartbeat and the following artifact in the EEG, which was calculated based on the entire dataset. For the calculation of the correction template, 21 pulse intervals were averaged.

Afterwards, the EEG was manually screened for artifacts, high-pass filtered (0.5 Hz), eye-blink/movement corrected using Independent Component Analysis (ICA), re-referenced to the average reference and segmented into epochs from 0 to 2 s post-CS (Mueller et al. 2014b). To ensure theta findings were not unduly affected by potential artifacts introduced by the ICA-based eye-movement correction, we performed an additional control analysis without ICA eye blink/movement correction. This control analysis included only epochs that were considered to be artifact-free, and the main results were confirmed (Supplementary Material C, see Supplementary Fig. S3A). Information on the residual number of trials per condition after artifact rejection is provided for both analyses (with and without ICA correction) in Supplementary Material D (see Supplementary Fig. S3B). To assess scalp power within the theta band (4–8 Hz; Mueller et al. 2014b) at frontal-midline channel Fz (Mueller et al. 2014b), Fast Fourier Transform (FFT) was applied (Hamming Window length: 10%). The estimated single-trial power was averaged across all trials for each CS and ln-transformed (Mueller et al. 2014a). For illustration purposes, the spectral line values were scaled as if they were calculated with a spectral line spacing of 1 Hz (i.e., μV^2/Hz). FER recall on Day 2 was assessed by comparing differential conditioned responses (CS+ vs. CS−) for nonextinguished and extinguished stimuli. Therefore, we computed a two-way repeated-measures ANOVA, including Contingency (CS+ vs. CS−) and Extinction Status (E vs. N) as repeated-measure factors.

Integration of fMRI and EEG Analyses

The primary goal of the present study was to bridge the gap between electrophysiological and hemodynamic correlates of FER, and to further integrate (1) theta oscillations on the one hand and (2) fear and extinction networks identified by fMRI on the other hand. To address this issue, we computed for each subject a score for theta power at frontal-midline channel Fz which reflects the degree of differential modulation to nonextinguished versus extinguished conditioned stimuli. As in our previous study (Mueller et al. 2014b), this FER score is computed as FER = (CS+N − CS−N) − (CS+E − CS−E). High FER scores indicate that differential fear responses with regard to theta power are higher for nonextinguished (CS+N − CS−N) compared with extinguished (CS+E − CS−E) stimuli during Day 2 recall. Thus, high FER scores are an indicator for successful recall of both conditioned fear (i.e., relatively larger fear response for nonextinguished stimuli) and extinguished fear (i.e., reduced fear

response for extinguished stimuli). In order to integrate fMRI and EEG findings for Day 2 recall, we computed simple regression analysis with theta FER scores (for each subject) as a covariate in the second-level group analysis. Regression analysis was performed with the BOLD response for the FER contrast representing recall of conditioned and extinguished fear as criterion, that is, [(CS+N – CS–N) vs. (CS+E – CS–E)]. For additional analyses on a trial-by-trial coupling of EEG theta oscillations and fMRI activation see Supplementary Material I.

Statistical Analyses

Except for fMRI data, which were analyzed in SPM12 (Wellcome Department of Cognitive Neurology, London, UK) as described above, statistical tests on other physiological data (EEG theta, SCRs) and subjective data (ratings of arousal, valence, and contingency awareness) were performed using SPSS 22 for Windows (IBM, Armonk, NY, USA). For statistical significance, $P \leq 0.05$ (two-sided) was required. For ANOVA analyses, significant interactions involving the factor Contingency (CS+ vs. CS–) were further analyzed using follow-up *t*-tests. As we had a priori hypotheses regarding the direction of the conditioned response (i.e., higher ratings of arousal and negative valence, larger SCRs, and higher theta power for CS+ relative to CS–), one-tailed paired-samples *t*-tests were used to compare CS+E/CS+N and CS–E/CS–N.

Results

Day 1 Fear Conditioning

CS evoked SCRs and affective CS ratings during the acquisition phase confirmed successful fear acquisition on Day 1. Figure 1C shows that during acquisition the 2 CS+ were associated with significantly higher SCR amplitudes than the 2 CS– (main effect of Contingency, $F(1,17) = 18.75$, $P < 0.001$). The absence of a significant Contingency × Later Extinction Status interaction ($F(1,17) = 1.13$, $P = 0.302$) confirmed that SCRs did not differ between to-be extinguished (CS+E = 0.10 ± 0.10; CS–E = 0.04 ± 0.05; $t(17) = 3.12$, $P = 0.003$, one-sided) and to-be nonextinguished (CS+N = 0.08 ± 0.05; CS–N = 0.04 ± 0.05; $t(17) = 4.13$, $P < 0.001$, one-sided) stimuli prior to the extinction phase.

Complementing these findings, relative to the CS–, the 2 CS+ were evaluated as significantly more unpleasant, more arousing, and more likely to be followed by a US after the acquisition phase (Fig. 1D,E). For negative valence ratings of the CS, the Contingency × Later Extinction Status × Time ANOVA revealed a significant interaction of Contingency and Time, $F(1,17) = 12.51$, $P = 0.003$. Both CS+ were rated as significantly more unpleasant after (main effect of Contingency, $F(1,17) = 10.56$, $P = 0.005$), but not prior to, the acquisition phase (main effect of Contingency, $F(1,17) = 2.52$, $P = 0.131$). There was no significant interaction involving the factor Later Extinction Status (Ps ≥ 0.115), indicating similar levels of conditioning for to-be extinguished (5-point scale after acquisition: CS+E = 4.11 ± 0.83; CS–E = 2.83 ± 0.99; $t(17) = 3.75$, $P = 0.001$, one-sided) and to-be nonextinguished (CS+N = 3.83 ± 1.20; CS–N = 2.89 ± 1.18; $t(17) = 2.15$, $P = 0.023$, one-sided) stimuli. For arousal ratings of the CS, a significant main effect of Contingency showed higher ratings for CS+ versus CS–, $F(1,17) = 5.07$, $P = 0.038$. Despite the absence of a Contingency × Time interaction, $F(1,17) = 1.36$, $P = 0.260$, results of separate two-way ANOVAs for each time point confirmed a significant conditioned response after (main effect of Contingency: $F(1,17) = 4.96$, $P = 0.040$), but not prior to the acquisition phase (main effect of Contingency: $F(1,17) = 0.70$, $P = 0.415$). CS+E (5-point scale: 3.28 ± 1.07; $t(17) = 1.87$, $P = 0.040$, one-sided) and CS+N (3.11 ± 1.08; $t(17) = 2.19$, $P = 0.022$, one-sided) were rated as significantly more arousing than CS–E (2.50 ± 1.10) and CS–N (2.28 ± 1.23) after fear acquisition. For CS–US contingency awareness ratings after fear acquisition, the Contingency × Later Extinction Status ANOVA revealed a significant main effect of Contingency, $F(1,17) = 185.68$, $P < 0.001$ (interaction Contingency × Later Extinction Status: $F(1,17) = 0.00$, $P = 1.000$). Contingency awareness of the CS–US relationship was similarly reliable for the to-be extinguished (4-point scale: CS+E = 1.89 ± 0.47; CS–E = 0.28 ± 0.46; $t(17) = 8.79$, $P < 0.001$, one-sided) and to-be nonextinguished stimuli (CS+N = 1.83 ± 0.38; CS–N = 0.22 ± 0.43; $t(17) = 13.63$, $P < 0.001$, one-sided).

Table 1 Localization and statistics of the peak voxels of significant activations for fear conditioning and extinction within previously defined ROIs (one-sample *t*-tests and correlations with EEG Theta FER[b])

Experimental phase	Brain Structure	Side	MNI coordinates X	Y	Z	t_{max}	P_{FWE}
Day 1 Acquisition							
CS+ unpaired > CS–	Insula	L	–38	20	–4	5.14	0.035*
CS+ unpaired < CS–	vmPFC	L	–10	52	–2	5.34	0.055+
Day 1 Extinction							
CS+E > CS–E			–No significant results–				
CS+E < CS–E	Hippocampus	L	–32	–24	–14	7.24	<0.001**
	Hippocampus	R	32	–18	–14	5.13	0.014*
Day 2 fear and extinction recall test, parametric modulation to account for amygdala habituation[a]							
(CS+N – CS–N) > (CS+E – CS–E)	Amygdala	L	–20	–8	–12	4.66	0.015*
Positive correlation with EEG Theta FER[b]	Amygdala	R	32	0	–22	4.72	0.015*
(CS+N – CS–N) < (CS+E – CS–E)[c]			–No significant results–				

[a]Weighted with an exponentially decaying function to model amygdala habituation.

[b]EEG Theta FER = frontomedial (electrode Fz) theta fear and extinction recall assessed by the tetrad contrast (CS+N – CS–N) – (CS+E – CS–E).

[c]Note that correlations of this BOLD contrast with EEG Theta FER scores are not displayed separately, as these correlations are already covered by the correlations listed above. For example, a positive correlation of Theta FER scores with the contrast (CS+N – CS–N) > (CS+E – CS–E) is equivalent to a negative correlation with the contrast (CS+N – CS–N) < (CS+E – CS–E).

+$P_{FWE} \leq 0.10$, *$P_{FWE} \leq 0.05$, **$P_{FWE} \leq 0.01$ (ROI analyses, FWE-corrected according to SPM12 small volume correction, one peak per cluster is listed). All coordinates (X, Y, Z) are given in MNI space. L = left, R = right brain hemisphere.

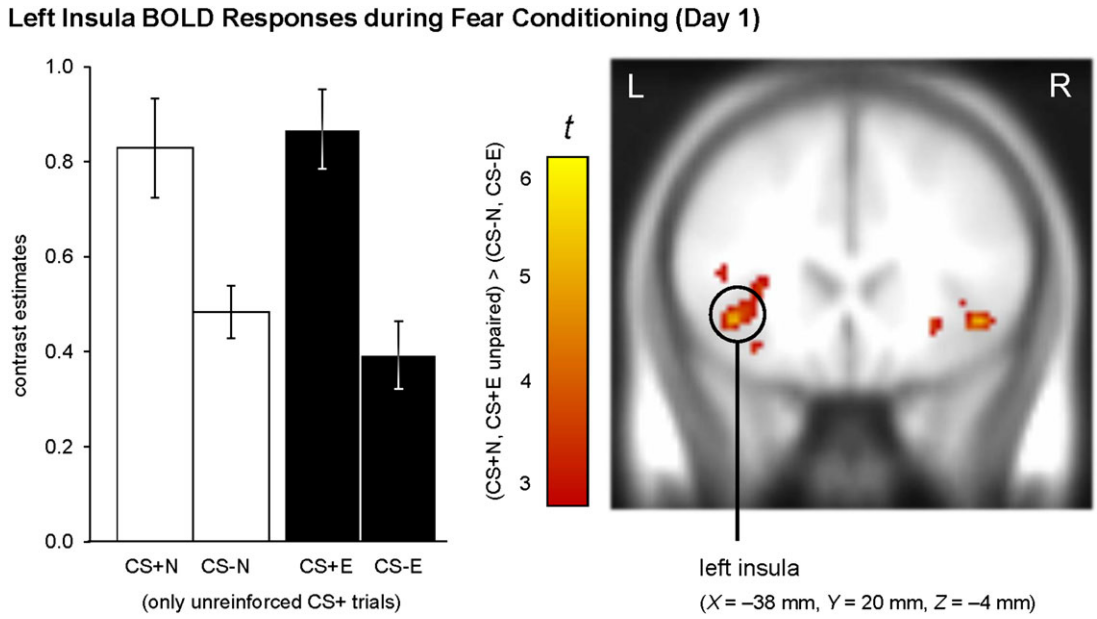

Figure 2. fMRI correlates of fear conditioning on Day 1. Insula activation was significantly enhanced for CS+ (CS+E, CS+N) compared with CS– (CS–E, CS–N). To confirm that neural responses did not differ between to-be extinguished and to-be nonextinguished stimuli, we constructed an additional first-level GLM which contained separate regressors for to-be extinguished and to-be nonextinguished CS+/CS–. Contrast estimates were extracted and subjected to a Contingency × Later Extinction Status ANOVA, which did not show a significant interaction, $F(1,17) = 0.65$, $P = 0.431$, but confirmed a significant main effect for Contingency, $F(1,17) = 24.45$, $P < 0.001$. For illustration purposes, the intensity threshold was set to $P \leq 0.005$ (uncorrected) with a minimal cluster threshold of $k \geq 5$ contiguous significant voxels. Activations (*t*-values) were superimposed on the MNI305 T1 template. All coordinates (*X*, *Y*, *Z*) are given in MNI space. L = left, R = right brain hemisphere. Bar graphs show the mean contrast estimates (±within-subject SEM, O'Brien and Cousineau 2014) for a cluster of voxels with $P \leq 0.005$ (uncorrected) surrounding the peak voxel within the insula ROI.

Collectively, these findings confirm successful fear conditioning at physiological and cognitive-affective levels. At the neural level, the left insula was the only region that was significantly more activated for CS+ versus CS– during the entire acquisition phase ($P_{FWE} = 0.035$, see Table 1 for statistical details). We confirmed that insula responses did not differ between to-be extinguished and to-be nonextinguished stimuli (Fig. 2).

Day 1 Fear Extinction

During subsequent Day 1 fear extinction, CS evoked SCRs showed a significant interaction of Contingency (CS+E vs. CS–E) and Time (beginning vs. end of extinction), $F(1,17) = 5.55$, $P = 0.031$. Participants showed higher SCRs for CS+E versus CS–E during the first 4 (Milad et al. 2013) extinction trials (CS+E = 0.16 ± 0.20; CS–E = 0.07 ± 0.11; $t(17) = 2.59$, $P = 0.010$, one-sided), indicating successful recall of conditioned fear at the beginning of the extinction session (Fig. 1C). Conversely, SCRs did not differ during the last 4 extinction trials (CS+E = 0.07 ± 0.12; CS–E = 0.06 ± 0.09; $t(17) = 0.14$, $P = 0.447$, one-sided), highlighting successful extinction learning. With respect to CS arousal ratings (Fig. 1D), a Contingency × Time (prior vs. after extinction) ANOVA revealed a significant main effect of Contingency, $F(1,17) = 5.17$, $P = 0.036$. As previously shown (Vansteenwegen et al. 2006), affective ratings remained relatively resistant against extinction throughout the entire extinction session. Differential arousal ratings were not reduced during extinction learning and indicated larger arousal ratings for CS+E versus CS–E both before (5-point scale: CS+E = 3.00 ± 0.91; CS–E = 2.44 ± 0.78; $t(17) = 1.97$, $P = 0.033$, one-sided) and after the extinction phase (5-point scale: CS+E = 2.94 ± 1.00; CS–E = 2.33 ± 0.84; $t(17) = 2.17$, $P = 0.023$, one-sided). For negative valence ratings (Fig. 1E), there was no significant effect of Contingency ($Ps \geq 0.104$). However, a significant effect of Time, $F(1,17) = 9.38$, $P = 0.007$, indicated a decline of ratings over time. Moreover, at the neural level, bilateral hippocampi were activated more strongly in response to CS–E versus CS+E throughout the entire extinction phase ($Ps_{FWE} \leq 0.014$, see Table 1).

Behavioral and SCR correlates of Day 2 Recall

While the Contingency × Extinction Status × Time interaction was only marginally significant with a two-sided test ($F(1,17) = 4.20$, $P = 0.056$; Fig. 1C), a planned contrast that reflected both habituation during recall and enhanced SCRs to CS+N versus all other stimuli (i.e., contrast values of 7, –1, –1, –1, … to CS+N, CS–N, CS+E, CS–E for the first and last 4 trials, respectively) was significant, $F(1,17) = 7.78$, $P = 0.013$. As expected, the nonextinguished previously conditioned fear-stimulus (CS+N = 0.18 ± 0.19) evoked larger SCRs than the nonextinguished CS– (CS–N = 0.09 ± 0.11; $t(17) = 2.10$, $P = 0.025$, one-sided) during the first 4 recall trials 24 h after conditioning and extinction, whereas there was no difference between CS+E (0.10 ± 0.17) and CS–E (0.11 ± 0.17) that had been presented during extinction, $t(17) = 0.30$, $P = 0.614$, one-sided (Fig. 1C). Moreover, there was no difference between CS+ and CS– during the last 4 recall trials for both extinguished (CS+E = 0.05 ± 0.07; CS–E = 0.05 ± 0.08; $t(17) = 0.002$, $P = 0.499$, one-sided) and nonextinguished stimuli (CS+N = 0.03 ± 0.06; CS–N = 0.05 ± 0.08; $t(17) = 1.08$, $P = 0.825$, one-sided). Furthermore, SCRs were larger during the first versus last 4 trials (main effect of Time, $F(1,17) = 5.04$, $P = 0.038$).

Moreover, similar to Day 1 extinction and in line with previous findings on extinction resistance of affective CS appraisal

(Vansteenwegen et al. 2006), both CS+ (5-point scale: CS+E = 3.03 ± 0.24; CS+N = 3.17 ± 0.20) stimuli were rated as significantly more arousing than both CS− stimuli (CS−E = 2.47 ± 0.20; CS−N = 2.39 ± 0.20) regardless of Day 1 extinction (Figure 1D; Contingency × Extinction Status × Time ANOVA; main effect of Contingency: $F(1,17) = 5.73$, $P = 0.029$; Contingency × Extinction Status interaction, $F(1,17) = 0.68$, $P = 0.420$). In addition to a main effect of Time, $F(1,17) = 7.56$, $P = 0.014$, the ANOVA on valence ratings did not show any significant main effects or interactions (Ps ≥ 0.101). Stimuli were rated as more negative at the beginning of Day 2 recall (Fig. 1E).

Electrophysiological Brain Correlates of Day 2 Recall

Replicating our prior human EEG study (Mueller et al. 2014b) and consistent with previous rodent work (Burgos-Robles et al. 2009), a significant Contingency × Extinction Status interaction, $F(1,17) = 6.88$, $P = 0.018$, revealed that differential (CS+ vs. CS−) frontomedial theta power was significantly reduced for extinguished versus nonextinguished stimuli (Fig. 3A). Moreover, we observed higher theta power for CS+N compared with CS−N, $t(17) = 2.31$, $P = 0.017$, one-sided, whereas there was no difference in theta power between extinguished stimuli CS+E and

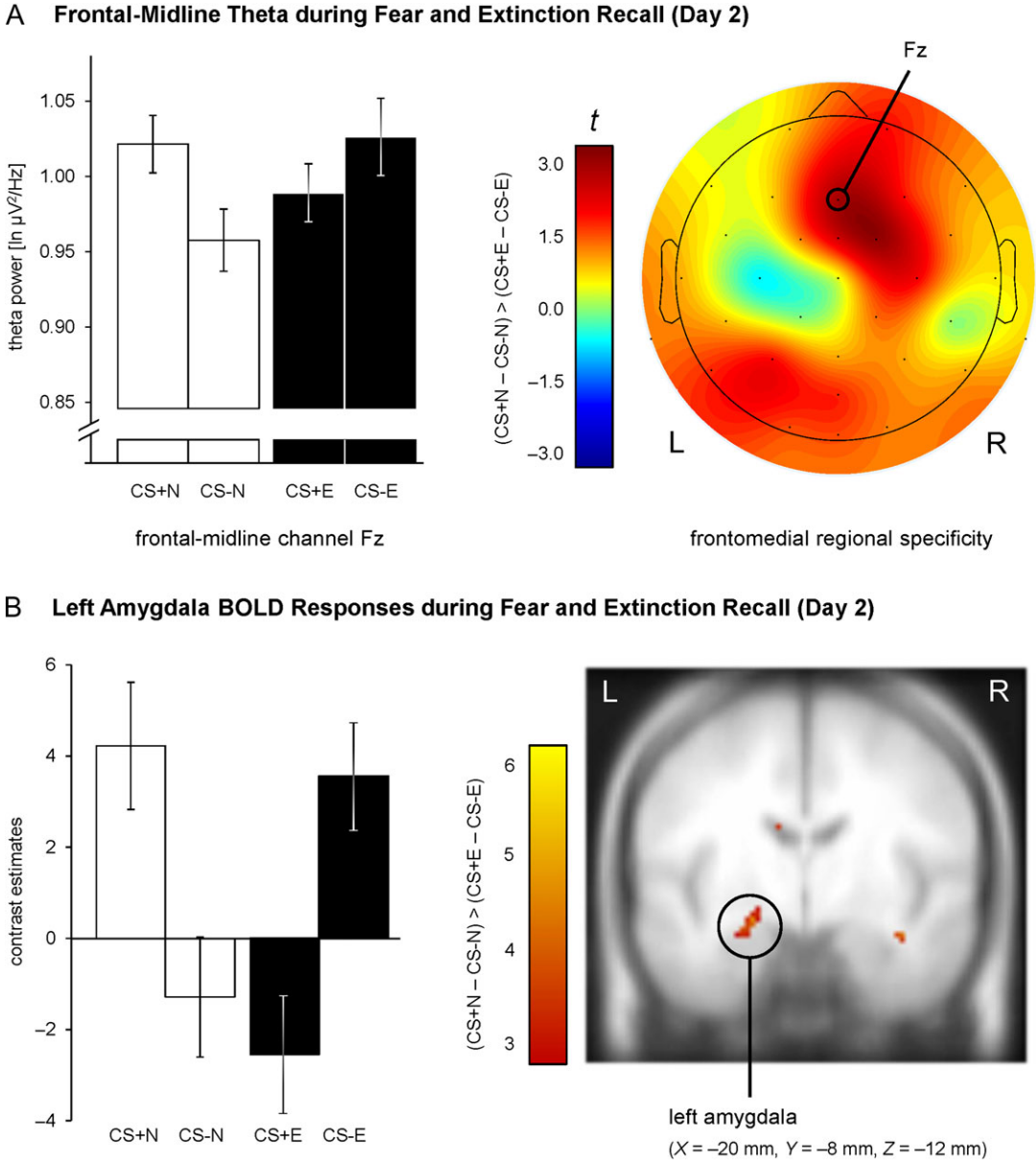

Figure 3. EEG and fMRI correlates of fear and extinction recall on Day 2. (*A*) Differential (CS+ – CS−) ln-transformed theta power at frontal-midline channel Fz was significantly reduced for extinguished versus nonextinguished stimuli (left). This effect was specific for frontomedial electrode channels (right). Bar graphs show the mean theta power (± within-subject SEM, O'Brien and Cousineau 2014). (*B*) Reduced differential amygdala responses (CS+ – CS−) for extinguished compared with nonextinguished stimuli. Habituation of amygdala activity was modeled by an exponentially decaying function, based on habituation of SCRs. For illustration purposes, the intensity threshold was set to $P \leq 0.005$ (uncorrected) with a minimal cluster threshold of $k \geq 5$ contiguous significant voxels. Activations (*t*-values) were superimposed on the MNI305 T1 template. All coordinates (X, Y, Z) are given in MNI space. L = left, R = right brain hemisphere. Bar graphs show the mean contrast estimates (± within-subject SEM, O'Brien and Cousineau 2014) for a cluster of voxels with $P \leq 0.005$ (uncorrected) surrounding the peak voxel within the amygdala ROI.

CS−E, $t(17) = 1.11$, $P = 0.859$, one-sided. Highlighting the specific role of frontomedial oscillations within the theta frequency band for fear expression (Mueller et al. 2014b), this effect showed a frontomedial topography (Fig. 3A) and was constrained to the theta frequency band (delta, 1–4 Hz: $P = 0.334$; alpha, 8–13 Hz: $P = 0.074$; beta, 13–30 Hz: $P = 0.242$).

Hemodynamic Brain Correlates of Day 2 Recall

During Day 2 recall, differential BOLD responses were significantly reduced for extinguished versus nonextinguished stimuli, that is [(CS+N − CS−N) > (CS+E − CS−E)], in the left amygdala (peak voxel in MNI space: $X = -20$ mm, $Y = -8$ mm, $Z = -12$ mm), indicating successful recall of conditioned and extinguished fear in this putative hub region of the fear network ($P_{FWE} = 0.015$, see Table 1 and Fig. 3B). A more detailed analysis of the activation time course (see Supplementary Material E) confirmed that this complex contrast of regressors, which were modeled by an exponentially decaying function, was primarily driven by increased amygdala activation to CS+N − CS−N (reflecting successful fear recall), and decreased amygdala activation to CS+E − CS−E (reflecting successful extinction recall) during the first trials of the recall phase, which diminished over time.

Reduced differential BOLD responses for extinguished versus nonextinguished stimuli was replicated in the bilateral amygdalae (left peak voxel: $X = -26$ mm, $Y = -8$ mm, $Z = -18$ mm; right peak voxel: $X = 30$ mm, $Y = -6$ mm, $Z = -20$ mm) when the analysis was restricted to the first 4 recall trials as in Milad et al. (2007b) ($Ps_{FWE} \leq 0.043$, Supplementary Material F, see Supplementary Table S1 for statistical details).

Integration of Electrophysiological and Hemodynamic Brain Correlates of Day 2 Recall

To investigate putative relations between EEG and fMRI data, a score reflecting the degree of differential modulation to nonextinguished versus extinguished conditioned stimuli [FER = (CS+N − CS−N) − (CS+E − CS−E)] was computed for theta power at frontal-midline channel Fz and entered as a covariate in second-level simple regression analysis. This analysis revealed a positive correlation between theta EEG FER and right amygdala (peak voxel in MNI space: $X = 32$ mm, $Y = 0$ mm, $Z = -22$ mm) BOLD FER modulation ($P_{FWE} = 0.015$, see Fig. 4 and Table 1). This indicates that high recall of conditioned and extinguished fear for EEG theta power (high FER theta scores) was associated with high FER for fMRI amygdala activation (high FER BOLD scores). Notably, 60% of the variance for the FER contrast was shared by theta oscillations and amygdala activation ($R^2 = 0.60$).

A trend for a similar theta-amygdala correlation for the FER contrast emerged ($P = 0.087$) when fMRI analysis was restricted to the first 4 (Milad et al. 2007b) recall trials (Supplementary Material F, see Supplementary Table S1). In addition, a negative correlation ($P = 0.038$) of theta EEG and vmPFC BOLD modulation emerged (Supplementary Material F, see Supplementary Fig. S5), consistent with a putative inhibitory role of the vmPFC on fear expression during early extinction recall (for a review, Milad and Quirk 2012).

The described covariation between theta power and amygdala activation is based on covariation of FER scores, which represent a combination of recall of conditioned and extinguished fear. Thus, it remains unclear whether this correlation of FER scores reflects covariation of fear-related (CS+N vs. CS−N) or extinction-related (CS+E vs. CS−E) effects. To disentangle these important alternative explanations, multivariate regression and univariate follow-up analyses were performed (Supplementary Material G). These analyses revealed that fear-related EEG theta modulations (CS+N − CS−N) negatively predicted extinction-related fMRI amygdala responses (CS+E − CS−E, $\beta = -0.74$, $P = 0.001$; Supplementary Material G, see Supplementary Table S2 for statistical details).

Discussion

Translating insights from rodent threat processing studies to human brains is both challenging and important, as assumed functional and structural homologies are controversial (Heilbronner et al. 2016). The primary goal of this study was to investigate the relationship between frontal theta oscillations and amygdala activation in the human brain, with emphasis on its specific role for fear and extinction memory. Therefore, we integrated (1) frontomedial theta oscillations during expression of conditioned fear and fear extinction, as previously revealed in human EEG and rodent studies, and (2) fear and extinction networks identified by fMRI in humans. Specifically, we recorded 31-channel EEG and fMRI simultaneously during a 24 h-delayed recall of previously conditioned as well as extinguished fear. As hypothesized, nonextinguished stimuli evoked stronger differential (CS+ vs. CS−) frontomedial oscillatory theta activity (EEG) and amygdala BOLD responses (fMRI) than extinguished stimuli. Furthermore, FER effects on EEG theta power covaried with amygdala responses, demonstrating for the first time that human frontomedial theta is linked to amygdala activation during threat processing, as previously observed in rodent studies (Gilmartin et al. 2014).

Successful fear conditioning on Day 1 could be shown at the psychophysiological (autonomic nervous system), subjective (arousal/valence), and neural (insula) level. Consistent with successful fear extinction, differential SCRs decreased during the extinction procedure and remained diminished even 24 h later. Similar to prior studies, bilateral hippocampal activation was increased for CS−E vs. CS+E during extinction learning, which was previously interpreted as indexing the development of an extinction memory trace (Phelps et al. 2004; Milad and Quirk 2012; Merz et al. 2014).

Replicating previous findings in humans (Mueller et al. 2014b) and in animals (Burgos-Robles et al. 2009; Gilmartin et al. 2014), extinction learning reduced differential frontal-midline theta power during extinction recall 24 h later. There is strong evidence from animal studies that medial frontal theta plays a critical role during sustained fear expression (Burgos-Robles et al. 2009) and extinction (Lesting et al. 2013). Complementing these animal findings, frontal-midline theta has also been linked to processing of fear and anxiety in humans (Mitchell et al. 2008; Mueller et al. 2014b; Cavanagh and Shackman 2015). Source-localization studies using EEG and MEG in humans have revealed that the AMC is the predominant generator for frontal-midline theta, which shows a reliable maximum at electrode Fz on the scalp level (Mitchell et al. 2008; Mueller et al. 2014b). Moreover, AMC-localized EEG activity is a predictor for subsequent heart rate changes (Panitz et al. 2013) with relevance for conditioned fear (Panitz et al. 2015). Collectively, these findings raise the possibility that AMC-mediated frontal-midline theta may play a crucial role in carrying out adaptive changes during fear expression (Cavanagh and Shackman 2015).

Mirroring findings involving theta oscillations, extinction training on Day 1 also reduced differential amygdala BOLD responses during Day 2 recall. While previous fMRI studies

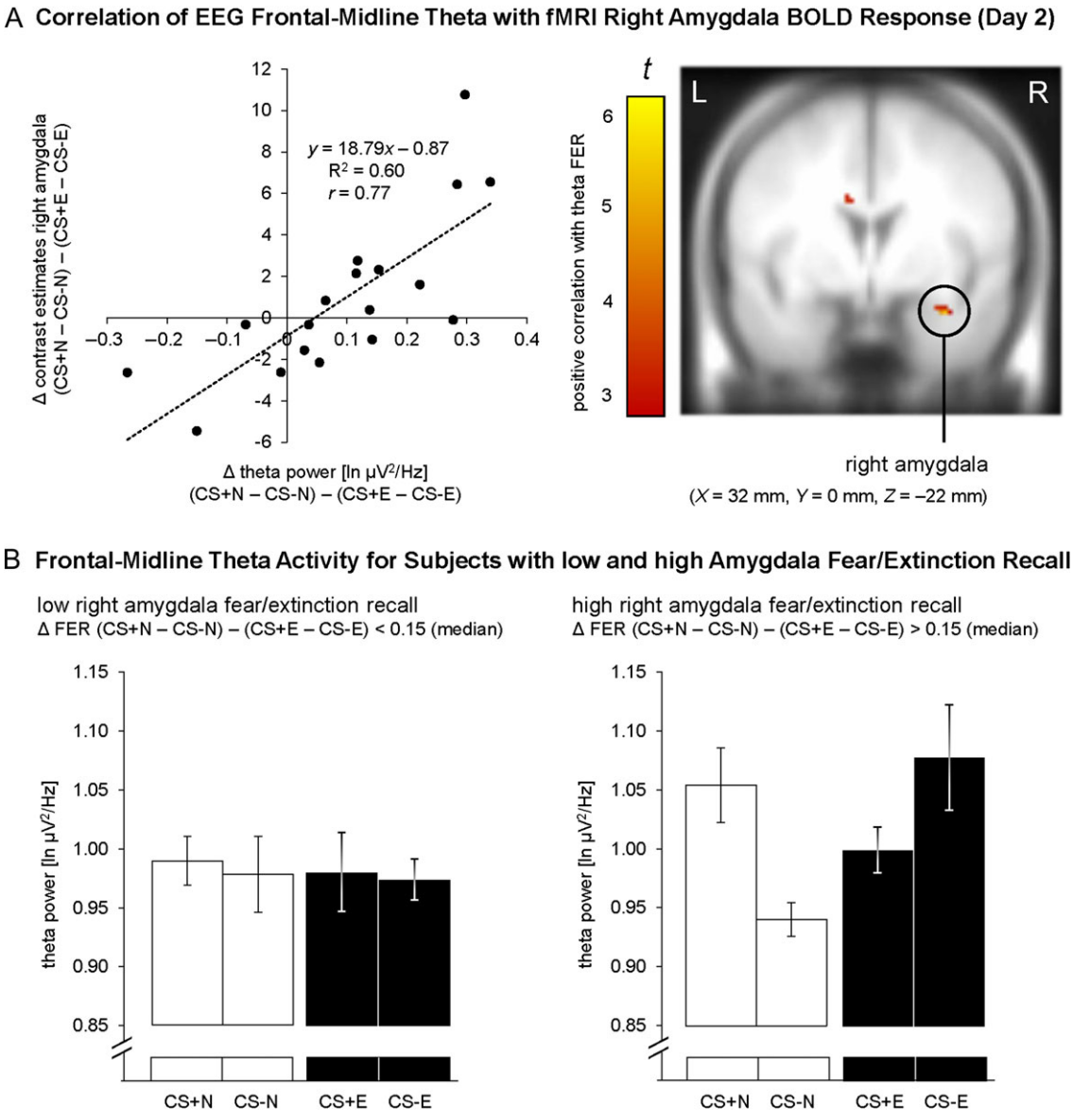

Figure 4. Integration of EEG frontomedial (Fz) theta power and fMRI right amygdala activation of fear and extinction recall on Day 2. (*A*) Positive correlation of theta modulations to conditioned and extinguished fear with BOLD responses in the right amygdala. Consistent with our assumed involvement of theta oscillations in AMC-amygdala connectivity (Gilmartin et al. 2014), this correlation indicates that subjects with relatively strong amygdala activation to nonextinguished (vs. extinguished) fear stimuli are characterized by relatively strong differential frontomedial theta power. For illustration purposes, the intensity threshold was set to $P \leq 0.005$ (uncorrected) with a minimal cluster threshold of $k \geq 5$ contiguous significant voxels. Activations (*t*-values) were superimposed on the MNI305 T1 template. All coordinates (X, Y, Z) are given in MNI space. L = left, R = right brain hemisphere. (*B*) To illustrate the positive correlation, right amygdala BOLD responses for the FER contrast (CS+N – CS–N) – (CS+E – CS–E) were compared based on median split, and theta power was assessed separately for subjects with low and high amygdala fear/extinction recall, that is, low/high FER BOLD scores (bar graphs show $M \pm$ within-subject SEM, O'Brien and Cousineau 2014). Higher differential theta power for nonextinguished versus extinguished CSs only emerged for subjects with high ($P < 0.001$), but not with low ($P = 0.929$) fear/extinction recall in the right amygdala.

have shown reduced amygdala activation to a previously extinguished CS+ compared with a CS– (Phelps et al. 2004; Hermann et al. 2016), the present study is the first to demonstrate that differential amygdala activation is significantly reduced for extinguished as compared with nonextinguished CS+ versus CS– stimulus pairs. Diminished amygdala activation during extinction recall may reflect processing of altered input about the predictive value of the CS+ (Phelps et al. 2004) and/or it may indicate a suppression of fear expression (Quirk and Mueller 2008), possibly through reduced afferent activity from the AMC (Vertes 2004) and/or increased inhibitory activity via connections from vmPFC to intercalated cells (Quirk and Mueller 2008; Pitman et al. 2012). This pattern of results is supported by animal findings suggesting that activity of fear-initiating amygdala neurons is "switched off" (Quirk and Mueller 2008) during the retrieval of extinction memories.

Importantly, the pattern of EEG theta power closely mirrored fMRI amygdala responses. Moreover, the differentiation between extinguished versus nonextinguished conditioned responses as measured by frontomedial theta scaled with the differentiation between extinguished and nonextinguished conditioned responses within the right amygdala. Notably, 60% of the variance in differential theta power could be explained by variation in differential amygdala BOLD responses. In particular, fear-related EEG theta

responses to nonextinguished stimuli covaried with fMRI amygdala activation to extinguished stimuli. This pattern was consistent with univariate analyses, where the Contingency × Extinction interaction on theta was primarily driven by nonextinguished CSs (mirroring Mueller et al. 2014b), whereas the Contingency × Extinction interaction on right amygdala was primarily driven by extinguished CSs (mirroring Phelps et al. 2004 and Hermann et al. 2016). This may reflect that communication within the fear and extinction network is modulated by synchronized theta oscillations (Bocchio and Capogna 2014; Gilmartin et al. 2014). In mice, theta synchronicity between the medial prefrontal cortex and the amygdala has been associated with better discrimination between CS+ and CS− after fear conditioning (Likhtik et al. 2014). Thus, our findings may reflect altered communication between the amygdala and prefrontal cortex (Likhtik and Gordon 2014) during the processing of threat-signaling stimuli that have not been subject to extinction.

As described above, the correlation with theta oscillations for fear compared with extinction recall was only evident for the right amygdala. Correlations between amygdala activation and other behavioral and physiological conditioned responses are often driven by the right amygdala (LaBar et al. 1998; Phelps et al. 2001, 2004) and right amygdala activation is particularly involved when the US elicits an immediate aversive response (Phelps et al. 2001), which might be particularly the case with electric shocks (LaBar et al. 1998). The present results suggest that immediate processing of aversive stimuli varied across participants, and that aversive responses generated by the right amygdala may be subject to top-down regulation by medial frontal theta oscillations emanating from the AMC (Gilmartin et al. 2014).

Taken together, our findings suggest that amygdala activation and AMC theta oscillations are both influenced by fear conditioning and extinction learning and that they covary with each other. Two routes are possible to explain functional coupling between both brain areas. First, the amygdala, as a hub for fear-related processes, may send efferent output during CS presentation to modulate the salience of the CS after extinction learning (Gilmartin et al. 2014; Senn et al. 2014). The AMC may then integrate these amygdaloid afferents with temporal and contextual information retrieved from other brain circuits (Fuster 2001; Gilmartin et al.2014). Second, reduced excitatory projections from the AMC back to the amygdala (Gilmartin et al. 2014) are presumed to come along with reduced theta synchrony (Bocchio and Capogna 2014). In the end, these projections may suppress amygdala-mediated fear responses (Pitman et al. 2012), signaling a reduced predictive value of the CS (Courtin et al. 2014; Gilmartin et al. 2014). Consistent with this second model, animal studies have shown that prelimbic theta input is crucial to reduce firing of amygdala neurons during safety (Likhtik et al. 2014), highlighting a dominant role of AMC theta for both fear and extinction learning (Quirk and Mueller 2008; Bocchio and Capogna 2014). In addition to the amygdala, the AMC may receive inhibitory inputs from the vmPFC, which is involved in extinction recall (Milad et al. 2007b; Hermann et al. 2016) and in the current study was negatively correlated with frontomedial theta (Supplementary Material F).

Some limitations of the study should be noted. First, in order to achieve an adequate signal-to-noise ratio for EEG recordings, it was necessary to include more trials than typically used in neuroimaging studies on fear conditioning and extinction (Fullana et al. 2016). Also, because BOLD responses in the amygdala have been found to show a rapid habituation after repeated CS presentations (Büchel et al. 1998), recall trials were weighted with an exponentially decaying function for the fMRI analysis (Büchel et al. 1998; Armony and Dolan 2001) but not the EEG analysis. Further, we implemented a complementary analytic approach that focused on only the first 4 fMRI trials (e.g., as in Milad et al. 2007b). Though this approach does not resolve the asymmetry of fMRI and EEG analyses, a very similar pattern of results emerged (see Supplementary Material F). Second, while the AMC is thought to be the predominant generator for frontal-midline theta oscillations (Mitchell et al. 2008; Mueller et al. 2014b), reduced differential EEG theta power was not accompanied by reduced differential AMC BOLD responses in our data. Fluctuations of the BOLD signal are challenging to directly map onto EEG power effects (Fellner et al. 2016). Although changes in EEG and fMRI signals are both correlated with local field potentials (Logothetis et al. 2001; Buzsáki, Anastassiou, Koch 2012; Herreras 2016), they may relate to different neural processes (Ekstrom 2010; Lopes da Silva 2013; Jorge, van der Zwaag, Figueiredo 2014), and the co-occurrence of multiple underlying physiological mechanisms is of particular relevance for measurable prefrontal brain correlates during threat processing (Etkin, Egner, Kalisch 2011; Delgado et al. 2016). Furthermore, it should be kept in mind that fear conditioning studies vary in the type of conditioned/unconditioned stimuli, number of trials, CS–US reinforcement rate, and CS–US delay, which seems to affect the degree with which AMC BOLD effects can be detected (Fullana et al. 2016). Third, the spatial resolution of noninvasive imaging methods used in humans, including fMRI, is limited (Keifer et al. 2015). In the present study, we found reduced differential amygdala activation after extinction training suggesting that measured amygdala activation was coding for the level of fear (Amano et al. 2011). Research in animals highlights the coexistence of both fear and extinction coding cells in the amygdala, with reciprocal patterns of activity (Quirk and Mueller 2008). To gain a better understanding of specific contributions of small adjacent subnuclei, connectivity of fear and extinction circuits within the amygdala with AMC theta oscillations should further be explored in animals.

Despite these limitations, this study demonstrated that simultaneous EEG–fMRI can capture oscillatory (theta) and subcortical (amygdala) fear-related activity at the same time in the human brain. With this approach, we linked animal-based findings on frontal theta to amygdala activity in humans. These findings lay the foundation for studying abnormal fear processing in psychopathology (Bowers and Ressler 2015). Given that current models imply exaggerated amygdala responses and deficient prefrontal functioning in patients with anxiety disorders (Bruhl et al. 2014; Rauch, Shin, Phelps 2006), investigations focusing on theta oscillations promise to be particularly important for probing disrupted communication among key nodes within the fear system. This knowledge might, in turn, open new avenues for treatment in anxiety and related disorders.

Authors' Contributions

M.F.J.S., C.P., A.H., C.H., D.A.P., and E.M.M. conceived and designed the study. M.F.J.S. and C.P. acquired the data. M.F.J.S., C.P., I.M.R., D.G.D., P.K., A.H., A.E.W., D.A.P., and E.M.M. analyzed the data. M.F.J.S., D.A.P., and E.M.M. drafted the article. All of the authors interpreted the data, discussed the results, and commented on the article.

Supplementary Material

Supplementary material is available at *Cerebral Cortex* online.

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Notes

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5 Empirical Studies: Supplementary Material for Manuscripts 1–6

5.1 Supplementary Material for Manuscript 1

Sperl, M. F. J., Panitz, C., Hermann, C., & Mueller, E. M. (2016). A pragmatic comparison of noise burst and electric shock US for fear conditioning research with many trials. *Psychophysiology*, *53*, 1352–1365. https://doi.org/10.1111/psyp.12677

Supplementary Table S1. Difficulty values (with standard deviations) and discrimination values of all items included in the self-constructed US questionnaire.

Item	Prior Expectation (before Habituation phase)		US Experiences (after Acquisition phase)	
The electric shock / white noise burst is …[b]	Item Difficulty ± *SD*	Item Discrimination[a]	Item Difficulty ± *SD*	Item Discrimination[a]
1. unpleasant	3.61 ± 0.92	.60	4.03 ± 1.17	.81
2. painful	2.58 ± 0.85	.48	2.84 ± 1.21	.68
*3. activating**	3.01 ± 0.89	.21	3.94 ± 1.34	.69
4. burdensome	2.81 ± 1.22	.80	3.10 ± 1.62	.90
5. annoying	3.71 ± 1.32	.75	4.00 ± 1.41	.78
6. surprising	4.26 ± 0.89	.82	4.39 ± 1.17	.67
7. straining	3.42 ± 1.20	.70	3.84 ± 1.42	.82
8. provoking	3.32 ± 1.25	.66	3.26 ± 1.73	.89
9. exhausting	3.42 ± 1.39	.82	3.39 ± 1.48	.80
10. irritating	3.23 ± 1.41	.85	3.23 ± 1.73	.90
11. seems physical harmful	1.58 ± 0.85	.49	1.81 ± 1.14	.48
*12. familiar to me**	2.23 ± 1.41	-.14	2.77 ± 1.26	.02
13. uncontrollable	3.16 ± 1.39	.39	3.48 ± 1.31	.30
14. frustrating	2.39 ± 1.26	.73	2.77 ± 1.56	.89

[a]Item Discrimination = part-whole-corrected item-total-correlation, i.e., correlation between each item and total score of the remaining items at one point of measurement (expectations/experiences)
[b]All items were rated on a 6-point Likert scale from 1 ("*not at all*") to 6 ("*extremely*").
*Asterisked items were excluded for calculating total scores due to low item discrimination coefficients.

5.2 Supplementary Material for Manuscript 2

Mueller, E. M., **Sperl, M. F. J.**, & Panitz, C. (2019). Aversive imagery causes de novo fear conditioning. *Psychological Science*, *30*, 1001–1015.
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Open Data and **Open Materials** available online at *Zenodo*:
https://doi.org/10.5281/zenodo.2591593

Supplementary Table S1. *F*-statistics, effect sizes and confidence intervals for significant ($p < .05$) and marginally significant ($p < .1$) ANOVA effects involving the factors *Cue Type* or *CS Type*

						95% CI of the pairwise difference		
Phase	***Dependent Variable***	***ANOVA Effect***	***F-value***[1)]	***p-value***	η^2_p	***aversive vs. neutral***	***aversive vs. nothing***	***neutral vs. nothing***
Acquisition	**Response to Cue**							
(Study 1)	Unpleasantness rating	Cue	219.00	.001	.83	**[4.90, 6.39]**	**[5.81**, 7.32**]**	[0.39, 1.46]
	IBI-A1	Cue	3.15	.048	.07	**[2.74, 16.66]**	[-14.14, 3.64]	[-2.96, 11.87]
	SCR	Cue	5.61	.010	.11	**[0.001, 0.030]**	**[0.002, 0.03]**	[-0.01, 0.01]
	Response to CS							
	Fear rating	CS Type	9.45	.001	.18	**[0.24, 0.96]**	**[0.33, 1.18]**	[-0.15, 0.47]
	Anger rating	CS Type	11.67	.001	.21	**[0.27, 0.97]**	**[0.37, 1.14]**	[-0.12, 0.39]
	Disgust rating	CS Type	5.08	.009	.10	[-0.16, 0.59]	**[0.20, 0.95]**	**[0.01, 0.72]**
	Arousal rating	CS Type	12.25	.001	.22	**[0.45, 1.09]**	**[0.34, 1.22]**	[-0.31, 0.33]
	Valence rating	CS Type	10.45	.001	.19	**[0.23, 1.01]**	**[0.49, 1.49]**	[-0.06, 0.80]
	IBI-D1	Block x CS Type	3.94	.023	.08	[-7.72, 6.00][3)] **[1.91, 17.60]**[4)]	[-3.44, 9.00][3)] [-4.31, 10.40][4)]	[-5.62, 12.90][3)] **[-12.95, -4.77]**[4)]
Extinction	Fear rating	CS Type	4.38	.016	.09	[-0.04, 0.55]	**[0.12, 0.77]**	[-0.09, 0.47]
(Study 1)	Anger rating	CS Type	7.43	.003[2)]	.14	**[0.19, 0.85]**	**[0.15, 0.90]**	[-0.22, 0.22]
	Disgust rating	CS Type	3.55	.033	.08	[-0.28, 0.52]	**[0.11, 0.87]**	[-0.01, 0.74]
	Arousal rating	CS Type	5.02	.010	.10	**[0.09, 0.80]**	**[0.12, 0.90]**	[-0.25, 0.39]
	Valence rating	CS Type	8.56	.001	.16	**[0.11, 0.90]**	**[0.43, 1.42]**	[-0.05, 0.87]
Acquisition vs. Extinction	Fear rating	Phase x CS Type	5.16	.008	.11	**[0.27, 1.07]**[4)] [-0.16, 0.48][5)]	**[0.27, 1.24]**[4)] **[0.04, 0.67]**[5)]	[-0.24, 0.42][4)] [-.09, .49][5)]
(Study 1)	Arousal rating	Phase x CS Type	3.84	.026	.08	**[0.54, 1.33]**[4)] **[0.07, 0.82]**[5)]	**[0.41, 1.41]**[4)] **[0.06, 0.87]**[5)]	[-.36, 0.32][4)] [-.37, 0.41][5)]
Acquisition	**Response to Cue**							
(Study 2)	Unpleasantness rating	Cue	163.22	.001	.80	**[5.07, 6.76]**	**[5.18, 7.01]**	[-0.32, 0.69]
	IBI-A1	Cue	5.18	.013[2)]	.12	**[-29.03, -2.38]**	**[-34.28, -3.96]**	[-12.55, 5.82]
	Response to CS							
	Arousal rating	CS Type	4.31	.017	.10	**[0.08, 0.91]**	**[0.07, 0.83]**	[-0.35, 0.25]
	Valence rating	CS Type	2.70	.074	.07	**[0.06, 1.11]**	[-0.27, 0.80]	[-0.80, 0.15]
	IBI-D1	Block x CS Type	2.76	.070	.07	[-8.70, 12.18][3)] [-3.38, 19.09][4)]	[-1.74, 15.19][3)] [-15.80, 9.26][4)]	[-4.00, 13.97][3)] [-22.34, 0.09][4)]
	Fear-potentiated startle	CS Type	3.51	.037	.11	[-0.20, 22.53]	**[1.67, 20.34]**	[-8.97, 8.65]
Extinction (Study 2)	Arousal rating	CS Type	6.08	.004	.14	**[0.17, 0.95]**	**[0.12, 0.83]**	[-0.39, 0.21]

Notes: Confidence intervals excluding zero (i.e., indicating a significant *t*-value for pairwise comparison) are in bold.
[1)] for all shown *F*-values, between-subjects degrees of freedom are df = 2 and within-subjects degrees of freedom of studies 1 and 2 are df = 88 and df = 80, respectively. Due to the exclusion of non-responders the within-subjects

degrees of freedom for fear-potentiated startle are df = 56; due to missing ratings of one participants in study 2, degrees of freedom for arousal and valence ratings in study 2 are df = 78. [2)] value after Greenhouse-Geisser correction; [3)] 95% CI for the first acquisition block, [4)] 95% CI for the second acquisition block, [5)] 95% CI for the second extinction block.

SCR: Additional Exploratory Analyses and Further Discussion

As suggested by a reviewer we performed additional analyses of SCR in which we (a) excluded individuals who showed no measurable SCRs (i.e., no SCRs > 0.01 µS) during acquisition, (b) removed the SCR to the first trial of acquisition (when no learning had yet occurred), (c) averaged SCRs for the remaining trials across three equally sized sets of trials (each set consisting of six trials for Study 1 and of three trials for Study 2 because trials with a startle burst were excluded), (d) performed a range correction for SCRs by dividing each single-trial SCR magnitude by the maximum response across all conditions (Lykken & Venables, 1971) and (d) specifically looked at SCR effects toward the middle and end of acquisition when learning effects should be more pronounced.

CS Type ANOVAs for the first set of trials (Study 1: $F(2,86) = 1.46$, $p = .24$; Study 2: $F(2,58) = .12$, $p = .89$), second set (Study 1: $F(2,86) = .28$, $p = .76$; Study 2: $F(2,58) = .416$, $p = .66$) and third set (Study 1*:* $F(2,86) = .76$, $p = .47$; Study 2: $F(1,58) = 1.72$, $p = .19$) bin did not reach significant main effects. This did not change when the general response magnitude was entered as a covariate. However, direct pairwise comparisons between the $CS+_{aversive}$ and the CS- yielded a significant *t*-value for the last acquisition trial set in Study 2, if one accepts one-sided testing, $t(29) = 1.83$, $p < .039$. A direct comparison between the $CS+_{aversive}$ and the $CS+_{neutral}$ was not significant, $t(29) = .44$, $p = .67$.

In general, we thus found no strong evidence for an effect of *CS Type* on SCR. This can be explained by the fact that SCR predominantly indicates contingency awareness rather than valence (Hamm & Weike, 2005) and that each of the three CSs (even the CS-) was followed by one of three different visual stimuli types (i.e., the "cues"). Participants may thus be similarly "contingency aware" for all three CS types and hence show similar responses. Note, that this aspect of the conditioning design is in contrast to "conventional" fear conditioning studies – in which the CS- is paired with no further stimulus – which typically find differences between CS+ and CS-.

IBI: Additional Analyses of Mean IBI

In addition to analyzing cardiac components D1, A1 and D2 in response to the CS, we also analyzed the mean IBI during the entire post-stimulus epoch (i.e., 7 s in Study 1 and 5 s in Study 2).

The *Block* x *CS Type* ANOVA in Study 1 yielded a marginally significant *Block* x *CS Type* interaction, $F(2,88) = 2.98$, $p = .06$. In the second block, the $CS+_{aversive}$ yielded stronger overall deceleration than the $CS+_{neutral}$, $t(44) = 2.29$, $p = .027$, and the $CS+_{neutral}$ yielded stronger acceleration than the CS-, $t(44) = 2.37$, $p = .022$. The *Block* x *CS Type* ANOVA in Study 2 yielded a non-significant *Block* x *CS Type* interaction, $F(2,88) = 2.30$, $p = .11$. In the second block, the $CS+_{aversive}$ yielded descriptively stronger overall deceleration than the $CS+_{neutral}$, $t(44) = 1.13$, $p = .27$ and the $CS+_{neutral}$ yielded descriptively stronger acceleration than the CS-, $t(44) = 1.58$, $p = .12$. While the *Block* x *CS Type* interactions were not significant for these analyses, the overall patterns are similar across studies and mirror the findings for the D1 component. They suggest that the fear bradycardia to the $CS+_{aversive}$ started within the first 1–2 s (i.e., during the D1 time window) and persisted throughout CS presentation. This time course of fear bradycardia mirrors fear conditioning studies with a physical aversive US (Panitz, Hermann, & Mueller, 2015; Panitz et al., 2018).

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5.3 Supplementary Material for Manuscript 3

Sperl, M. F. J., Wroblewski, A., Mueller, M., Straube, B., & Mueller, E. M. (2021). Learning dynamics of electrophysiological brain signals during human fear conditioning. *NeuroImage*, *226*, 117569. https://doi.org/10.1016/j.neuroimage.2020.117569

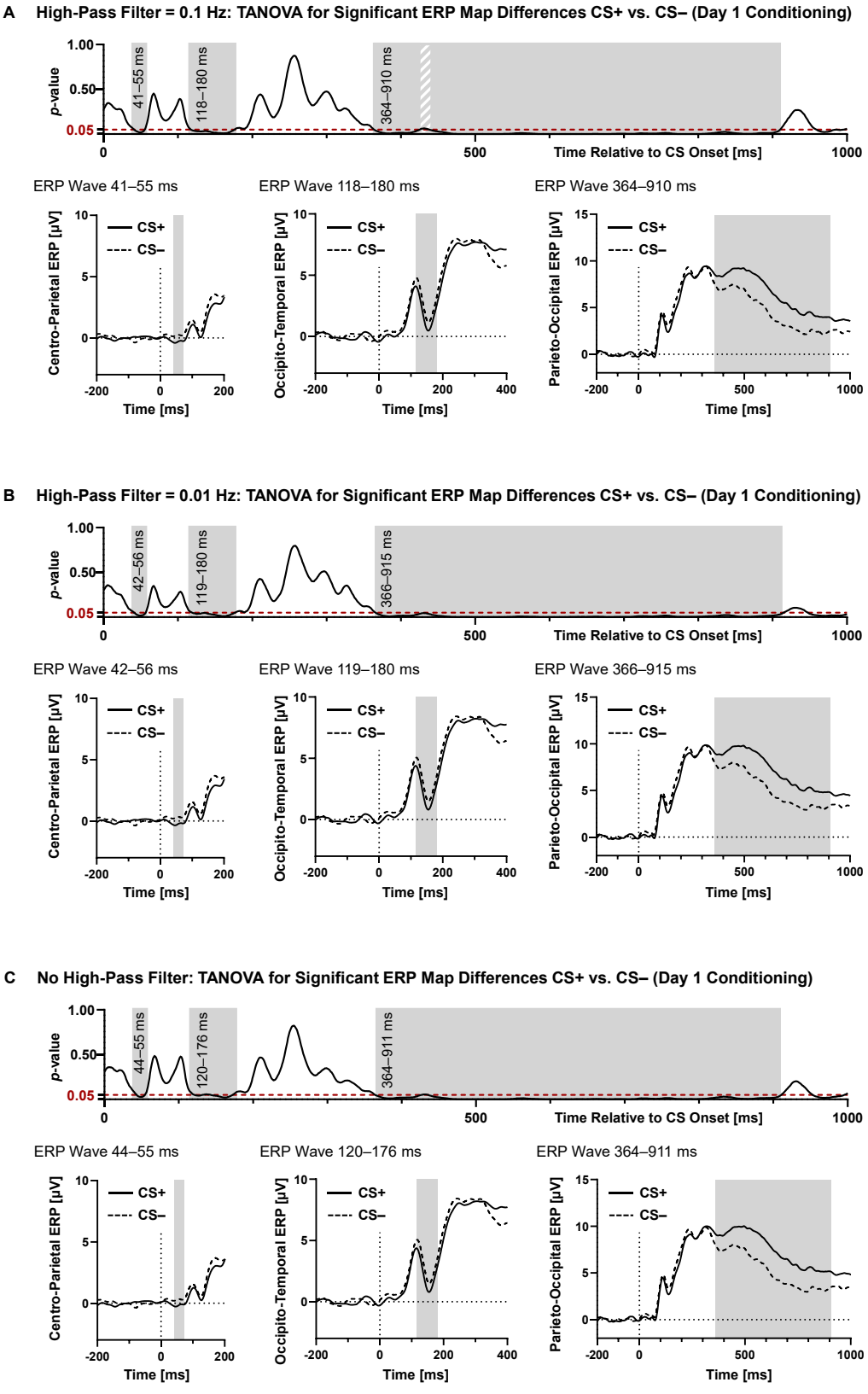
Open Data and **Open Materials** available online at *Zenodo*:
https://doi.org/10.5281/zenodo.4294603

A Control EEG Analysis with Different High-Pass Filter Settings

As described in the *Materials and Methods* section of the manuscript (see section 2.7.), raw EEG data were high-pass filtered with a cutoff frequency of 0.5 Hz (signal amplitude is attenuated by 3 dB at cutoff frequency, 4th order Butterworth filter, 24 dB/octave roll-off). The high-pass filter was applied to remove slow drifts, which can be caused by skin potentials (Cohen, 2014). In the present study, we were specifically interested in revealing the learning curve of ERP effects, which requires averaging across a relatively *low* number of trials. Thus, our goal was to maximize the signal-to-noise ratio at the single-trial level. The significance of signal-to-noise ratio for choosing appropriate high-pass filter cutoffs has recently been discussed in the literature (Maess et al., 2016a, 2016b; Tanner et al., 2016). In fact, Kappenman and Luck (2010) demonstrated that the application of a 0.5 Hz high-pass filter can substantially reduce the number of trials that are required to average for detecting a significant effect between ERP amplitudes. This finding is of particular relevance for our study, as our goal was to identify changes across trials. However, others have shown that high-pass filtering (especially ≥ 0.5 Hz) can affect early ERP responses (Acunzo et al., 2012; Tanner et al., 2015) and attenuate the amplitude of slower ERP components (Duncan et al., 2009; Hajcak et al., 2012).

To ensure that our findings are not unduly affected by filter settings, we performed additional control analyses with 0.1 Hz, 0.01 Hz, and no high-pass filters. It is crucial to emphasize that independent component analysis (ICA) requires high-pass filtering ≥ 0.5 Hz to obtain reliable and valid decomposition results (Winkler et al., 2015; Dimigen, 2020). Therefore, we used an approach which has previously been described by Debener et al. (2010) and Winkler et al. (2015). Specifically, the ICA weights for all analyses were trained on 0.5 Hz high-pass filtered data. Afterward, ICA matrix files were exported and the "learned" weights ("IC filters") were used to unmix and back-project the unfiltered, 0.01 Hz filtered, and 0.1 Hz filtered data.

Importantly, these additional control analyses demonstrated the robustness of all findings regardless of filter settings: Each of the described analyses confirmed significant ERP effects in similar time windows (see Supplementary Figure S1). First, for each filter cutoff frequency, TANOVA identified a short-latency period (i.e., < 100 ms) with continuously significant differences between CS+ and CS- maps (0.5 Hz filter: 33–60 ms; 0.1 Hz filter: 41–55 ms; 0.01 Hz filter: 42–56 ms; no high-pass filter: 44–55 ms). Second, TANOVA identified a mid-latency period which was similar for each filter cutoff frequency (0.5 Hz filter: 108–200 ms; 0.1 Hz filter: 118–180 ms; 0.01 Hz filter: 119–180 ms; no high-pass filter: 120–176 ms). Third, TANOVA identified a long-latency period (0.5 Hz filter: 468–820 ms; 0.1 Hz filter: 364–910 ms; 0.01 Hz filter: 366–915 ms; no high-pass filter: 364–911 ms). Taken together, results were similar and robust across high-pass filter settings. These additional analyses highlight the validity of our findings described in the *Results* section of the manuscript (section 3.).



Supplementary Figure S1. The EEG processing steps described in the manuscript involved a 0.5 Hz high-pass filter to remove slow drifts and enhance statistical power for analyses on learning curves which are based on a relatively low number of trials. To ensure that our findings are not unduly affected by filter settings, we performed additional control analyses with (A) 0.1 Hz, (B) 0.01 Hz, (C) and no high-pass filters. Importantly, all analyses revealed similar findings.

B Residual Number of Trials per CS Condition after Artifact Rejection

The residual number of trials after artifact rejection was similar across CS+ and CS- conditions and is shown in Supplementary Figure S2.

C TANOVA on Amplitude-Normalized Maps (GFP = 1)

Significant map differences between conditions can be produced (1) by a change in strength of similar generators ("quantitative difference of activation"), (2) by differences in source orientation or distribution ("qualitative difference"), or (3) by a combination of both (Koenig and Melie-García, 2009; Koenig et al., 2011; Michel and Murray, 2012; Habermann et al., 2018). In the present study, we were interested in both quantitative and qualitative differences between maps. Thus, our TANOVA approach tested for both effects, which offers the possibility to detect *all* (i.e., strength- *and* topography-related) systematic electrocortical differences between CS+ and CS- (Maurer et al., 2005; Michel and Murray, 2012).

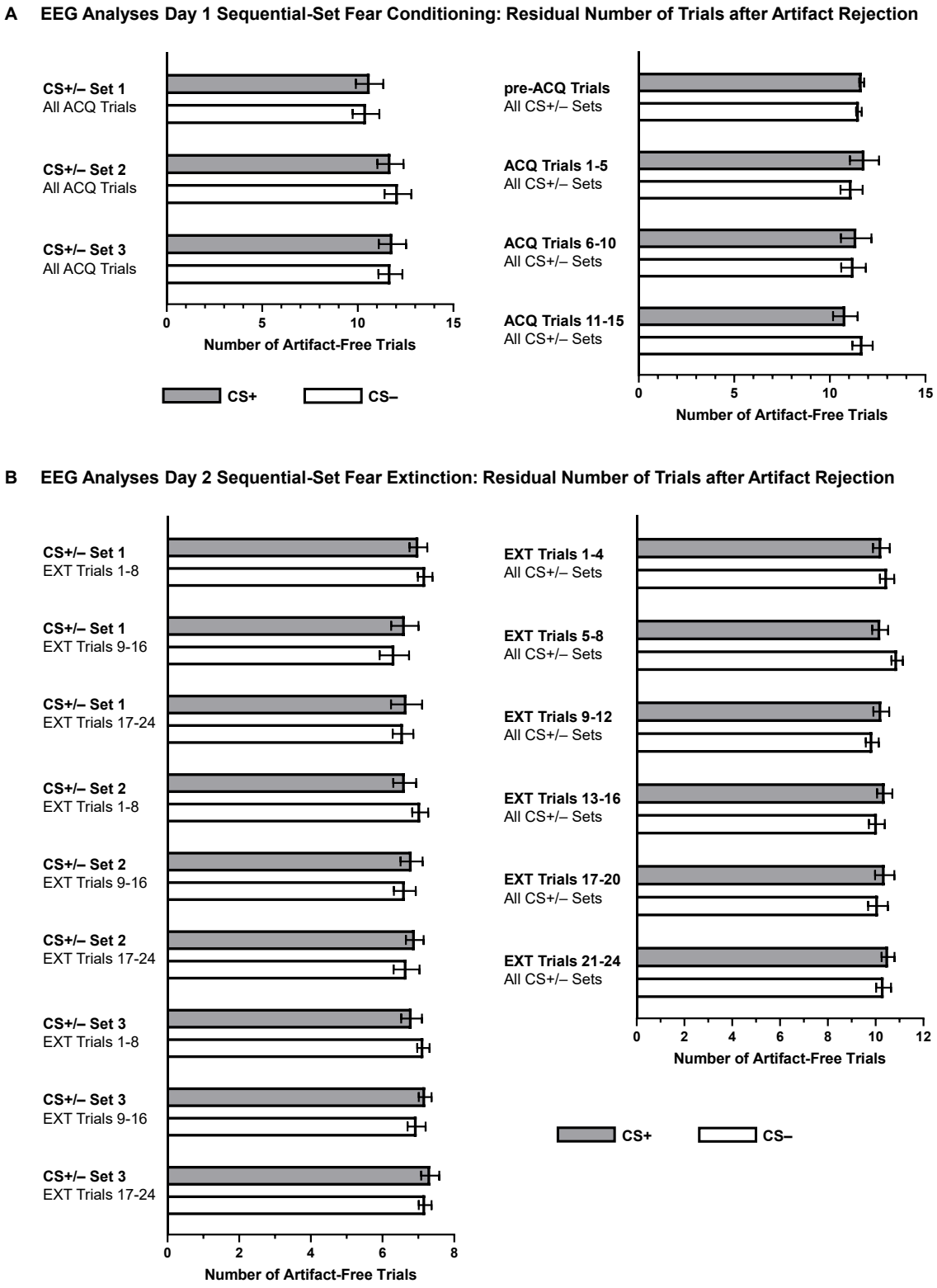
As described in the *Results* section 3.3., we found significant differences during three distinct time windows: (a) 33 to 60 ms, (b) 108 to 200 ms, and (c) 468 to 820 ms after CS onset. Supplementary Figure S3 shows the voltage maps separately for CS+ and CS-. In addition, the grand-grand average ERPs (across CS+ and CS- trials and stimulus sets) at centro-parietal electrode channels (CP1, CPz, CP2, P1, Pz, P2, POz), occipito-temporal channels (T7, C5, TP7, CP5, P7, P5, PO7, PO3, T8, C6, TP8, CP6, P8, P6, PO8, PO4), and parieto-occipital channels (P1, Pz, P2, PO3, POz, PO4, O1, Oz, O2) are displayed.

Notably, Murray et al. (2008) suggested that stimuli of negative emotional valence may be processed through a more efficient neural circuit, which would imply the contribution of (at least partially) different generators. Thus, in addition to the TANOVA approach described above, we also computed a second TANOVA on the amplitude-normalized maps (Koenig and Melie-García, 2009; Koenig et al., 2011; Habermann et al., 2018). To achieve data normalization, all potential values of a specific map were divided by its Global Field Power (GFP), i.e., all maps were scaled to have GFP = 1. The GFP, which is calculated as the mean absolute potential difference in the field, represents the spatial standard deviation across all electrodes at a specific time point and is considered to be a reference-free measure of response strength (Lehmann and Skrandies, 1980).

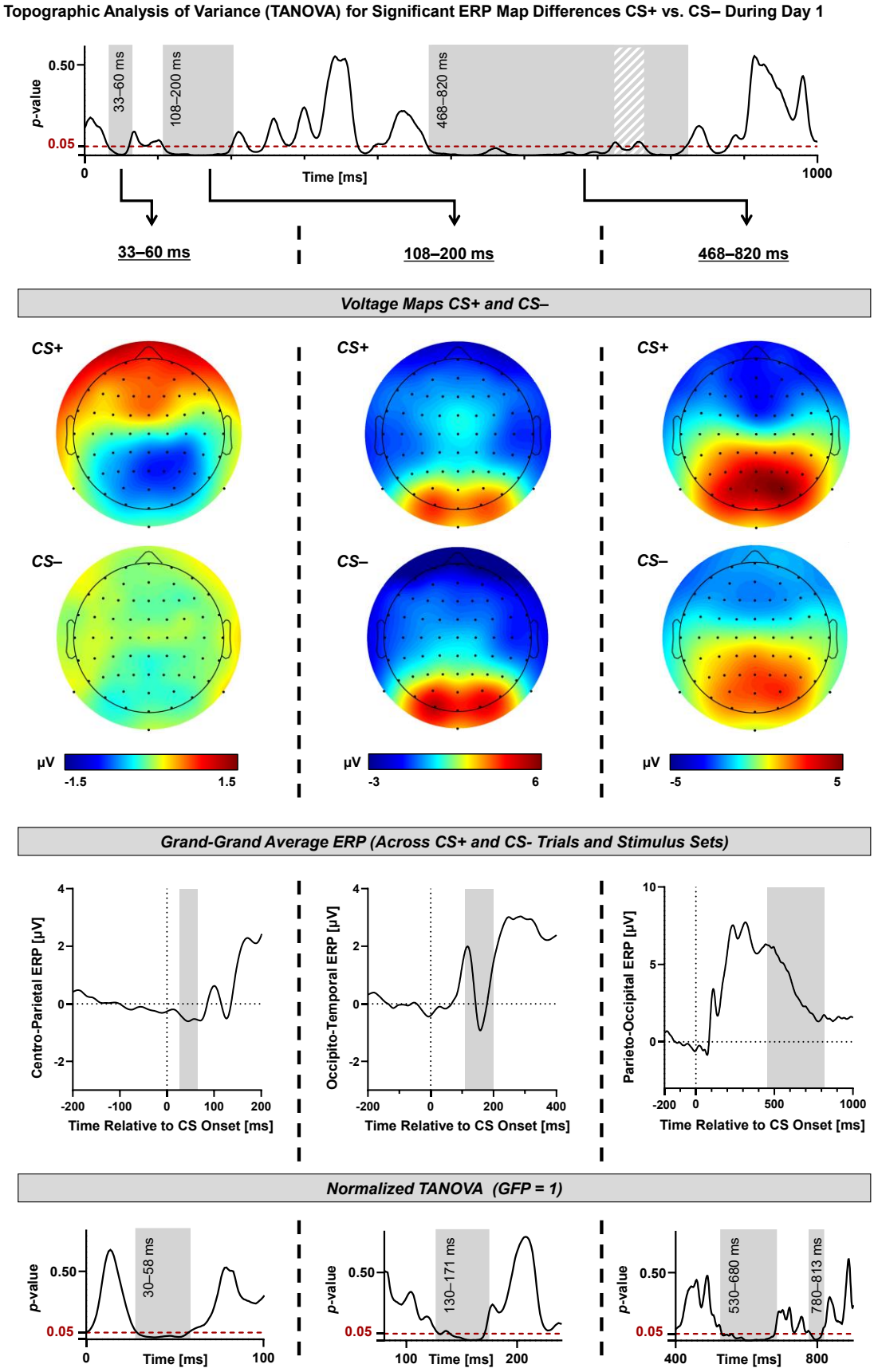
Importantly, the TANOVA on the amplitude-normalized maps indicated that different intracranial brain generators contributed to the effects in each of the three time windows during fear acquisition (see Supplementary Figure S3, lower panel). Both analyses (i.e., with and without normalization) revealed similar time windows, although periods of contiguous time points with significant TANOVA results ($p \leq .05$) were slightly shorter if maps were amplitude-normalized to GFP = 1. To explicitly assess the degree of agreement between conclusions from both TANOVA approaches (with and without normalization), we dichotomized both "*p*-curves" (i.e., $p > .05$ = "0" ["not significant"], $p \leq .05$ = "1" ["significant"] for each sampling point), calculated Cohen's Kappa, and confirmed moderate agreement between both analyses ($\kappa = .47$, $p < .05$).

D TANOVA for Sequential-Set Fear Extinction on Day 2

For day 2 fear extinction, we expected a large conditioned response during the first trials and a decline toward later trials (see section 3.4. of the manuscript). Hence, ERPs from only the first extinction training block (i.e., the first 8 trials) from all three stimulus sets were averaged for the extinction TANOVA (see Supplementary Figure S4). Importantly, this analysis indicated significant differences between ERP maps for CS+ and CS- in the time window from 460 to 730 ms after CS onset (averaged across the significant time window: TANOVA $p < .001$). An additional TANOVA on the amplitude-normalized maps (GFP = 1) confirmed that different intracranial brain generators were involved.

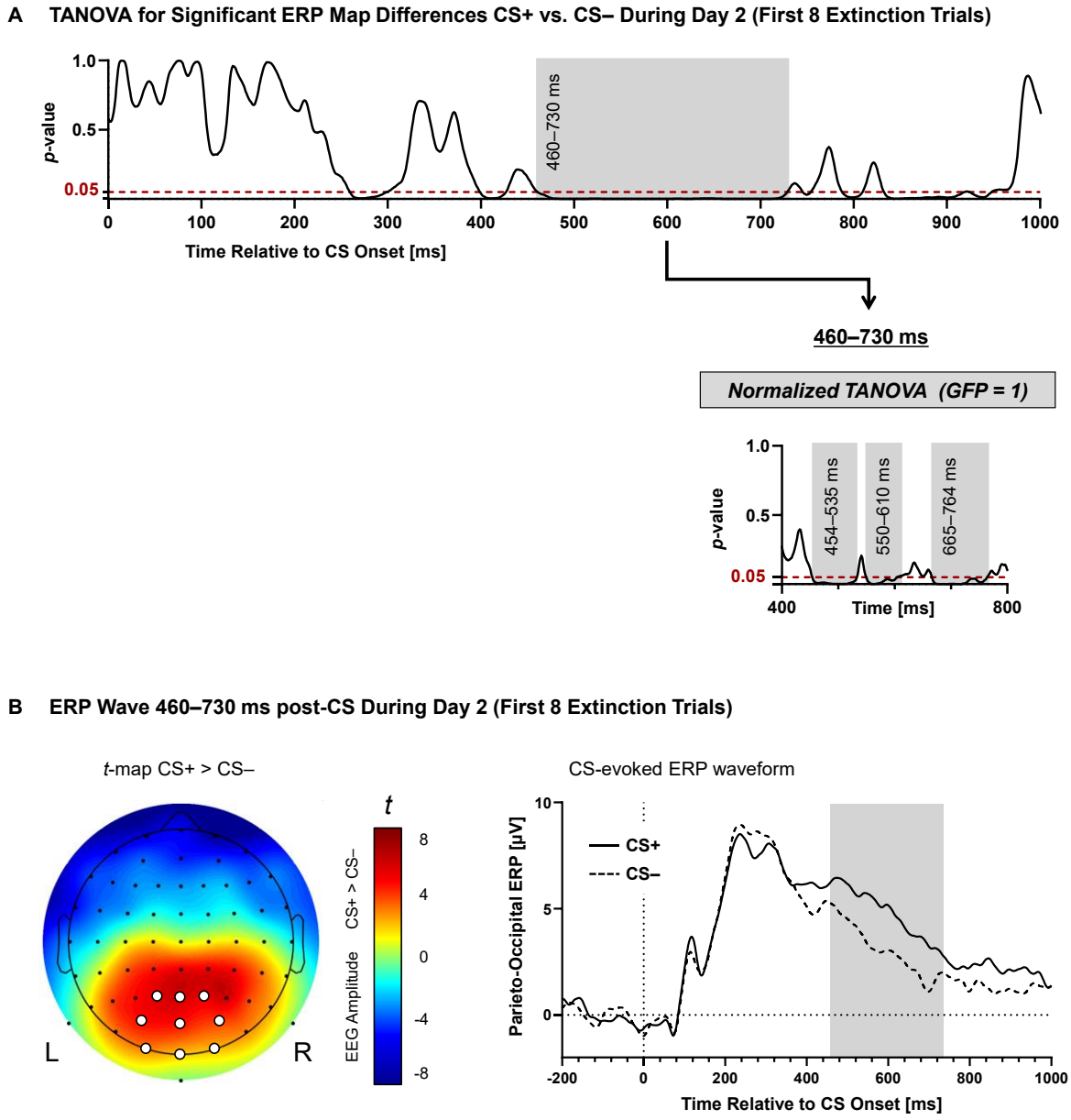


Supplementary Figure S2. Number of artifact-free trials (M ± *SEM*) for (A) day 1 conditioning and (B) day 2 fear extinction. The left panels show the residual number of trials for EEG analyses reported in the *Results* sections 3.3. and 3.4. (i.e., similar conditioned responses for CS+/CS- sets 1, 2, and 3). In addition, the right panels show the residual number of trials for EEG analyses reported in the *Results* section 3.5. (i.e., learning dynamics of ERP effects, as revealed by block-wise analyses after averaging across CS+/CS- sets). Importantly, the residual number of trials was similar across CS+ and CS- conditions.



Supplementary Figure S3. The TANOVA for the fear acquisition stage showed map differences during three time periods, which may be explained by quantitative or qualitative differences of

activation. For each time window, voltage maps are presented separately for CS+ and CS-. In addition, we show the grand-grand average ERP (across CS+ and CS- trials and stimulus sets) at centro-parietal electrode channels (CP1, CPz, CP2, P1, Pz, P2, POz), occipito-temporal channels (T7, C5, TP7, CP5, P7, P5, PO7, PO3, T8, C6, TP8, CP6, P8, P6, PO8, PO4), and parieto-occipital channels (P1, Pz, P2, PO3, POz, PO4, O1, Oz, O2). To explicitly assess whether differences in source orientation or distribution gave rise to these effects, we calculated an additional TANOVA on GFP = 1 normalized data. Importantly, this additional analysis indicated that different intracranial brain generators contributed to the effects in each of the three time windows, which was corroborated by a moderate agreement between both TANOVA analyses (as indicated by Cohen's Kappa, $\kappa = .47$, $p < .05$).



Supplementary Figure S4. Event-related potential (ERP) responses evoked by CS+ compared with CS- during day 2 sequential-set fear extinction. (A) The topographic analysis of variance

(TANOVA) indicated that topographic maps were significantly different for CS+ compared with CS- during the 460–730 ms period (i.e., *p*s ≤ .05, gray-shaded area). Note that there were also two earlier periods with *p*s ≤ .05 (260–300 and 400–425), but these were too short to reach overall significance according to global duration statistics. An additional TANOVA on the amplitude-normalized maps (GFP = 1) yielded similar results. (B) During the 460–730 ms period, the ERP amplitude at parieto-occipital channels was significantly more positive for CS+ versus CS- (left panel). To visualize ERP waveforms (right panel), the electrode sites P1, Pz, P2, PO3, POz, PO4, O1, Oz, and O2 were averaged (channels are shown as white dots in the t-map, significant effects could be confirmed at all electrode sites). The gray-shaded area in panel B indicates the measurement window for ERP amplitudes. "L" = left hemisphere, "R" = right hemisphere.

E Additional ERP Analyses for Sequential-Set Fear Extinction on Day 2

As described in sections 3.3. and 3.4. of the manuscript, we computed separate TANOVAs for the acquisition and extinction stages. Both experimental phases were assessed on two different days. It has been shown that sleep is critical for the consolidation of conditioned fear (Menz et al., 2016), and can also affect neural signatures of fear recall (Pace-Schott et al., 2015). To account for possible influences of sleep on consolidation of neural fear responses, a separate TANOVA was carried out for extinction training on day 2.

However, we also performed additional analyses for the extinction stage using the same time windows (and channels) that were derived from the acquisition TANOVA. Analyses for the early (33–60 ms; all *p*s ≥ .194) and mid-latency (108–200 ms; all *p*s ≥ .079) periods did not reach significance, indicating no significant differences between CS+ and CS- on the extinction day during the day 1 time windows. Converging with the results on learning dynamics reported in the *Results* section 3.5., the analysis on the late-latency period supported successful extinction learning also when the time window was derived from the acquisition TANOVA (468–820 ms). Notably, the *Contingency* x *Sub-Block* ANOVA yielded a linear trend for the *Contingency* x *Sub-Block* interaction ($F(1,20) = 7.45$, $p = .013$).

To statistically justify claims about the development and change of involved neural processes between the acquisition and extinction parts of the experiment, we also included the test day as additional within-subject factor for the analysis on the 468–820 ms period. This *Contingency* x *Sub-Block* (day 1: four pre-acquisition trials, last five acquisition trials; day 2: first four extinction trials, last four extinction trials) x *Test Day* (day 1 acquisition, day 2 extinction) ANOVA revealed a significant *Contingency* x *Sub-Block* x *Test Day* interaction ($F(1,20) = 19.59$, $p < .001$). As expected, differential fear responses (CS+ vs. CS-) increased during fear acquisition training (*Contingency* x *Sub-Block* interaction: $F(1,20) = 19.73$, $p < .001$), and decreased during fear extinction training (*Contingency* x *Sub-Block* interaction: $F(1,20) = 6.71$, $p = .017$).

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5.4 Supplementary Material for Manuscript 4

Sperl, M. F. J., Panitz, C., Skoluda, N., Nater, U. M., Pizzagalli, D. A., Hermann, C., & Mueller, E. M. (submitted). Alpha-2 adrenoreceptor antagonist yohimbine potentiates consolidation of conditioned fear. Submitted to *Neuropsychopharmacology*.

Open Data and **Open Materials** will be available online at *Zenodo* after acceptance.

A Exclusion Criteria for Participants

As described in the *Materials and Methods* section of the main text, we recruited 55 healthy male students at Justus Liebig University Giessen. One participant did not complete the study. Three subjects were excluded as they fulfilled our criterion of "unlikely explicit contingency awareness" (i.e., higher awareness ratings for CS- than CS+ after acquisition, as defined by [1]). Therefore, the final sample consisted of $N = 51$ participants ($n = 17$ yohimbine group, $n = 16$ sulpiride group, $n = 18$ placebo group). There were no significant group differences in age, body mass index (BMI), self-reported sleep quality measures (nights before day 1 and day 2), and trait/state anxiety (see Supplementary Table S1). All subjects were males, right-handed, and between the ages of 18 and 35 (mean age = 22.61 years, $SD = 3.05$ years). Exclusion criteria were (1) habitual use of tobacco, anorectics, or any illegal or prescription drugs; (2) BMI < 17 or > 30; and (3) a history of neurological or cardiovascular diseases (e.g., hypertension or coronary heart disease), metabolic disorders, gastric or duodenal ulcers, gastrointestinal tract bleedings, hepatic or kidney diseases, or other chronic diseases that would require individual medical clarification. Participants underwent a standardized clinical interview (Short Version of the Diagnostic Interview for Mental Disorders, Mini-DIPS [2]) to confirm the absence of mental disorders. In addition, participants were asked to refrain from alcoholic or caffeinated drinks, tea, juice, chewing gum, and strenuous exercise prior to the experiment [3,4]. All subjects gave written informed consent to participate. They received monetary compensation (€10 per hour) or course credit.

B Conditioned and Unconditioned Stimuli

Conditioned Stimuli. Four different black-and-white male faces [7] with a neutral expression were used as CSs. The faces were assigned to CS types (i.e., CS+E, CS+N, CS-E, CS-N) in a counterbalanced fashion. During each trial, the CS face was presented for 4 s with a size of 13 x 18 cm^2 on a black background (22-inch monitor, about 0.80 m from participant), using the computer program Presentation 17.0 (Neurobehavioral Systems, Berkeley, CA, USA). Prior to each trial, a white fixation cross was presented for 1 s. During a jittered intertrial interval (defined as CS offset to CS onset) of 6–8 s, a black screen was shown. As part of a habituation phase, which was performed prior to the acquisition phase, each CS was shown 5 times.

Unconditioned Stimulus. We used a 95 dB(A) white noise burst (duration: 1 s) as US, which has previously been shown to elicit a reliable conditioned response for the present paradigm [8]. The white noise burst started 3 s after CS onset and was presented by a room speaker. If the 95 dB(A) burst was experienced as too loud, the sound pressure level was reduced to 92 dB(A) (one participant each in the yohimbine and sulpiride groups and two participants in the placebo group). The sound pressure level was measured at the participant's head position (approximately 2.30 m from the speaker).

Supplementary Table S1. Sample Characteristics: Age, Body Mass Index (BMI), Sleep, and Trait/State Anxiety Measures (Mean ± Standard Deviation). There were no significant differences between the three experimental groups.

Variable	Yohimbine Group *n* = 17 *M* (± *SD*)	Sulpiride Group *n* = 16 *M* (± *SD*)	Placebo Group *n* = 18 *M* (± *SD*)	Between-Groups Statistical Comparison
Age and Body Mass Index (BMI)				
Age [Inclusion Criterion: 18–35 years; Actual Age Range: 18–32 years]	22.24 (± 2.75) 19–28	22.63 (± 3.24) 18–29	22.94 (± 3.28) 19–32	$F(2,48) = 0.23$, $p = .796$
BMI [Inclusion Criterion: 17–30 kg/m²; Actual BMI Range: 17.73–29.94 kg/m²]	23.65 (± 3.13) 19.25–29.32	24.22 (± 3.20) 17.73–29.73	23.86 (± 2.22) 20.99–29.94	$F(2,48) = 0.17$, $p = .846$
Sleep measures[1]				
Sleep Quality Before Day 1 [1–5]	3.76 (± 0.75)	3.69 (± 0.60)	3.89 (± 0.58)	$F(2,48) = 0.42$, $p = .661$
Sleep Quality Before Day 2 [1–5]	4.29 (± 0.69)	4.50 (± 0.73)	4.11 (± 0.76)	$F(2,48) = 1.22$, $p = .306$
Hours Slept Before Day 1	6.47 (± 1.10)	6.70 (± 1.20)	6.53 (± 1.09)	$F(2,48) = 0.18$, $p = .837$
Hours Slept Before Day 2	7.71 (± 0.94)	8.28 (± 1.06)	7.78 (± 1.32)	$F(2,48) = 1.28$, $p = .288$
Tiredness Day 1 [1–4]	1.53 (± 0.51)	1.25 (± 0.45)	1.50 (± 0.51)	$F(2,48) = 1.58$, $p = .216$
Tiredness Day 2 [1–4]	1.12 (± 0.33)	1.06 (± 0.25)	1.11 (± 0.32)	$F(2,48) = 0.16$, $p = .853$
Trait and State Anxiety[2]				
STAI Trait Anxiety [20-80]	37.41 (± 9.19)	35.69 (± 5.49)	33.50 (± 5.76)	$F(2,48) = 1.37$, $p = .265$
STAI State Anxiety Day 1 [20–80]	31.53 (± 3.95)	33.38 (± 3.22)	34.83 (± 6.21)	$F(2,48) = 2.16$, $p = .126$
STAI State Anxiety Day 2 [20–80]	29.29 (± 3.57)	30.63 (± 4.94)	32.78 (± 7.97)	$F(2,48) = 1.58$, $p = .217$

[1]Sleep quality and quantity for the preceding night were assessed on both days at the beginning of the experiment. Participants were asked to indicate subjective sleep quality on a 5-point Likert scale (1 = "very bad sleep"; 5 = "very good sleep") and sleep quantity (i.e., the number of hours they slept). In addition, subjective tiredness was measured on a 4-point Likert scale (1 = "not tired at all"; 4 = "very tired").

[2]Trait (assessed on day 1) and state anxiety (assessed on both days, at the beginning of the experiment) was measured using the German version (Laux, Glanzmann, Schaffner, & Spielberger, 1981 [5]) of the State Trait Anxiety Inventory (STAI; Spielberger, Gorsuch, & Lushene, 1970 [6]). The range of possible STAI scores varies from 20 ("minimal") to 80 ("maximal intensity of anxiety").

C Pharmacological Challenge: Yohimbine, Sulpiride, and Placebo

As explained in the main text, participants received in a double-blind manner an oral dose of either 10 mg of yohimbine HCl, 200 mg of sulpiride, or a placebo pill. The capsules were compounded by the study pharmacist and were identical in appearance. A cup of water was provided along with the capsules. To control for potential pharmacodynamic or pharmacokinetic food-drug interactions [9], participants received a standardized breakfast between day 1 acquisition and extinction phases. On day 2, participants were asked not to eat for two hours before the experiment.

Yohimbine [10] and sulpiride [11] are generally well-tolerated, and adverse side effects are very rare. Following previous studies [12–15], we used a single acute dose of 10 mg yohimbine hydrochloride (HCl), which is rapidly absorbed and reaches peak plasma levels within 1 h [16,17]. The elimination half-life ranges from 0.25 to 2.5 h. However, an active yohimbine metabolite (11-hydroxy-yohimbine) shows similar α2 adrenoceptor antagonist properties [17,18] and exhibits a longer half-life of around 6 h [19,20]. This may explain the relatively long-lasting pharmacodynamic effects. Sulpiride is only slowly absorbed from the gastrointestinal tract; peak plasma levels occur within 3–4 h, and the average elimination half-life ranges from 3–10 h [21–23].

Following the recommendations by Crockett and Fehr [24], we asked participants at the end of day 1 to report their beliefs about whether they had received an active substance (yohimbine or sulpiride) or a placebo pill. The proportion of participants who said that they had received a placebo (yohimbine group: 41%; sulpiride group: 50%; placebo group: 50%) did not differ between groups ($X^2(2) = 0.35$, exact $p = .881$). This indicated successful blinding.

D Salivary α-Amylase

Yohimbine and sulpiride were administered to enhance noradrenergic and dopaminergic transmission, respectively. To confirm the active effect of yohimbine on central noradrenaline release [25–27], we measured salivary α-amylase activity (sAA). Saliva samples were collected by using the passive drool method on both days at several time points (day 1: 9:30 AM, 11:30 AM, 11:57 AM, 12:07 PM, 12:17 PM, 12:27 PM, 12:37 PM, 1:15 PM, 2:15 PM; day 2: 3:00 PM; see Figure 2 in the main text). Prior to each saliva collection time point, participants were instructed to rinse their mouths with water and to swallow all saliva. Afterward, participants were asked to collect passively the newly produced saliva in their mouths for two minutes and to release the cumulated saliva into a plastic sample tube (SaliCap Set; IBL International, Hamburg, Germany). The specimens were stored at –20 °C until assay. After thawing for biochemical analysis, samples were centrifuged for 11 min at 3,000 rpm, resulting in a clear supernatant. Saliva was diluted 1:400 using 0.9% saline solution. Next, sAA activity was measured using a kinetic colorimetric test and reagents obtained from Roche (Roche Diagnostics, Mannheim, Germany). The intra- and inter-assay coefficients of variance were less than 10%. To correct for skewed distributions, sAA data were log10-transformed. The sAA data of four participants could not be analyzed because the values were below the detection limit (< 3 U/ml; $n = 1$ in the placebo group) or because the values were extremely high (> 800 U/ml; $n = 1$ in the placebo group; $n = 2$ in the sulpiride group).

E Affective CS Ratings

Participants were asked to rate each CS with regard to its associated arousal (1 = “not arousing”; 5 = “very arousing”) and valence (1 = “very pleasant”; 5 = “very unpleasant”), prior to and after each experimental stage. After acquisition, we also assessed the subjective awareness of the CS–US contingency (0 = “CS was never followed by US”; 3 = “CS was always followed by US”).

F Skin Conductance, Electrocardiogram (ECG), and Electroencephalogram (EEG)

Skin conductance, ECG, and EEG were recorded at 1,000 Hz using a QuickAmp 72 amplifier (Brain Products, Munich, Germany). The monitor delay (33 ms) was assessed with a Brain Products Photo Sensor, and all marker latencies were corrected accordingly. All physiological data were low-pass filtered online with a cutoff frequency of 200 Hz. Preprocessing was performed in BrainVision Analyzer 2.1.2 (Brain Products, Munich, Germany).

Skin Conductance Responses (SCRs). To assess electrodermal activity (exosomatic measurement, 0.5 V direct current), two Ag/AgCl electrodes of a 10 mm diameter filled with isotonic (0.5% NaCl) electrolyte medium were placed on the thenar/hypothenar sites of the left hand. The raw signal was low-pass filtered (1 Hz, signal amplitude was attenuated by 3 dB at cutoff frequency, 4th order Butterworth filter, 24 dB/octave roll-off) offline in BrainVision Analyzer 2.1.2 (Brain Products, Munich, Germany) and downsampled to 100 Hz. For visual data inspection, artifact correction, and trough-to-peak analyses, the skin conductance data were exported to Ledalab 3.4.9 [28,29], which was implemented in the MATLAB 9.2 environment (MathWorks, Natick, MA). Technical artifacts were corrected with spline or cubic interpolation. Next, a skin conductance response (SCR) score was calculated for each trial. This was defined as the amplitude-sum of significant SCRs within 1 and 5 s after the CS onset. SCRs during the first second after CS onset were omitted [30], and SCRs smaller than 0.01 µS were considered to be zero responses. Before averaging, SCR scores were logarithmized, ln(µS+1), to ensure a normal distribution. Afterward, SCR scores for each CS type were averaged across trials. For the acquisition stage, only unreinforced CS+ trials were included to avoid contamination by an evoked response to the US.

Evoked Heart Period (HP). The electrocardiogram (ECG) was measured with pre-gelled Ag/AgCl disc surface electrodes (F-55 type, Megro, Wesel, Germany) in the Lead II configuration (right arm and left leg, ground electrode on left arm). The raw ECG data were band-pass filtered (1−30 Hz, signal amplitude was attenuated by 3 dB at cutoff frequencies, 4th order Butterworth filter, 24 dB/octave roll-off) and notch filtered (50 ± 2.5 Hz, 16th order Butterworth filter, 96 dB/octave roll-off) offline. Next, R-spikes were detected automatically using the ECG Markers Solution implemented in BrainVision Analyzer 2.1.2 (Brain Products, Munich, Germany). After manual screening, trials with artifacts were rejected and R-spike latencies were corrected if necessary. One participant had to be excluded from ECG analyses for the acquisition stage due to heavy recording artifacts. After artifact correction, a continuous heart period trace was calculated using custom-made MATLAB scripts (MATLAB 9.2; MathWorks, Natick, MA). In particular, we converted the ECG to a time course of interbeat intervals (IBIs). Afterward, each IBI time point reflected the latency between the pre- and succeeding R-spike in ms [31]. This IBI time series was then segmented into epochs ranging from –1 to 8 s relative to the CS onset, baseline-corrected (1 s pre-CS), and averaged across trials for each CS type. Fear conditioning is typically associated with a robust cardiac deceleration for CS+ compared with CS- [32–34], which usually overlaps with the US presentation [33,35]. Consistent with previous studies [36,37], the mean heart period change from 2 to 5 s after CS onset was extracted for statistical analyses. For the acquisition stage, only unreinforced CS+ trials were analyzed. To achieve a sufficient signal-to-noise ratio for EEG recordings on the second day [38], we presented a large number of CS trials (60 trials per CS type). Because of a rapid habituation of fear-conditioned SCRs [1] and bradycardia [37], peripheral measures of fear and extinction recall on day 2 were assessed during the first ten trials.

Electroencephalography (EEG). The electroencephalogram (EEG) was recorded with a 64-channel actiCAP active electrode system and actiCAP electrode caps (Brain Products, Munich, Germany), referenced against the average. Raw EEG data were high-pass filtered (0.1 Hz, signal amplitude was attenuated by 3 dB at cutoff frequency, 4th order Butterworth filter, 24 dB/octave roll-off) and notch-filtered (50 ± 2.5 Hz, 16th order Butterworth filter, 96 dB/octave roll-off) offline. Ocular artifacts (eye blinks and movements) were corrected with independent component

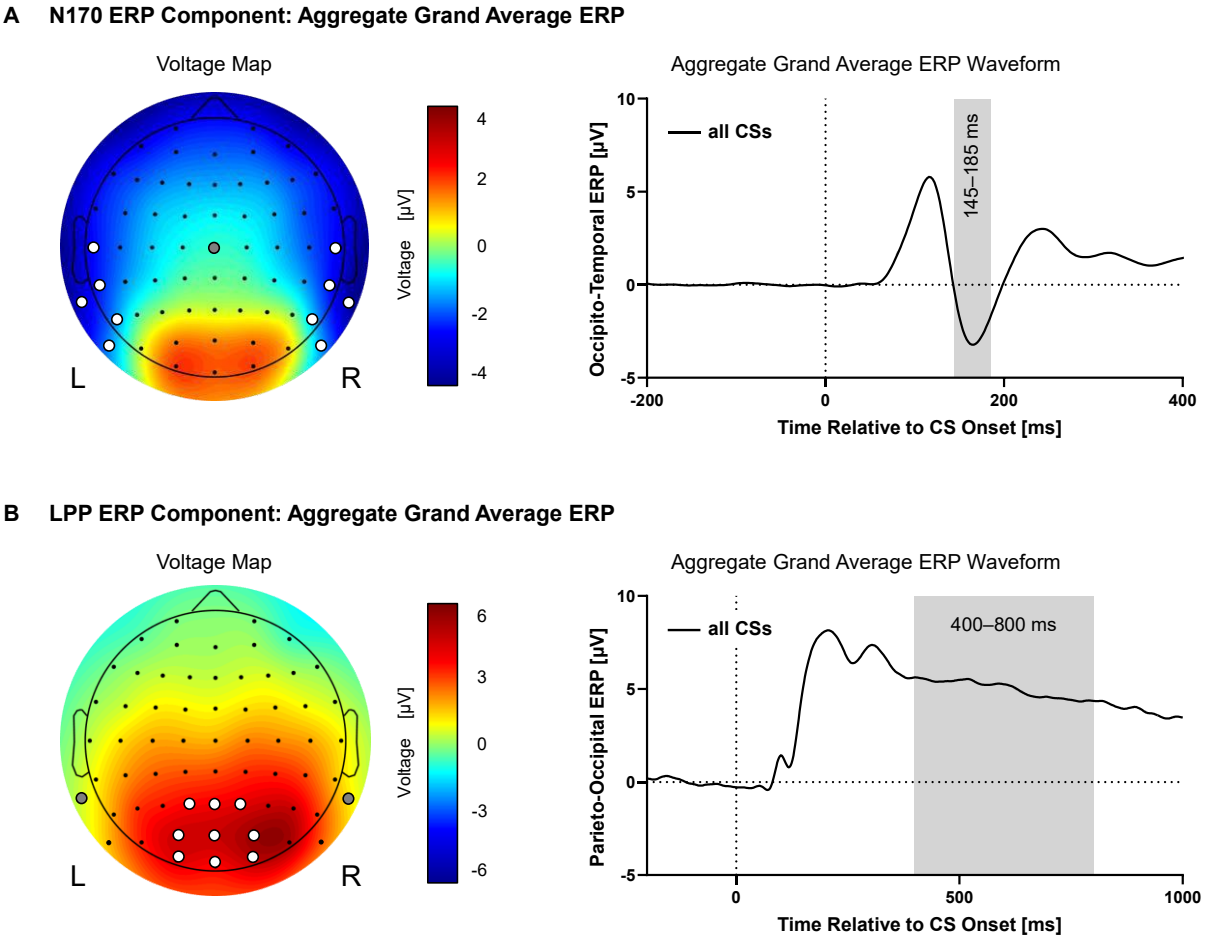
analysis (extended infomax ICA with classic principal component analysis sphering on the whole artifact-free EEG dataset). To obtain reliable and valid decomposition results [39,40], the raw EEG signal was 0.5 Hz high-pass filtered for ICA only. Specifically, ICA weights were trained on the 0.5 Hz high-pass filtered data, ICA matrix files were exported, and, afterward, the "learned" weights ("IC filters") were used to unmix and back-project the 0.1 Hz filtered EEG data [39,41]. All EEG data were manually screened for artifacts. Intervals that contained artifacts in at least one channel were excluded from further analyses, and corrupted channels were interpolated (spherical spline [42]). Finally, EEG was downsampled to 500 Hz. Prior to N170 and LPP analyses, a 30 Hz low-pass filter was applied (signal amplitude was attenuated by 3 dB at cutoff frequency, 4th order Butterworth filter, 24 dB/octave roll-off).

N170 ERP Component. For analyses of the N170 component, the EEG was referenced against Cz, as this central reference better highlights the N170 at occipito-temporal channels [43], which is hypothesized to be generated primarily in the fusiform gyrus [44]. Next, ERPs were segmented relative to the CS onset (–200 ms to 400 ms) and baseline-corrected. As expected, the aggregate grand average ERP [45] (collapsed across trials of all CS types, across all experimental groups, and across day 1 extinction and day 2 recall) showed a distinct negativity at bilateral occipito-temporal sites during the typical N170 period (Supplementary Figure S1A). Consistent with previous research [46,47], this negativity was particularly pronounced at T7, TP7, TP9, P7, and PO9 over the left hemisphere, and at T8, TP8, TP10, P8, and PO10 over the right hemisphere. The aggregate grand average pooled across these channels showed a negative peak at 165 ms after CS onset. Consequently, we used the mean voltage during the time window from 145 to 185 ms (i.e., the negative peak ± 20 ms) for statistical analyses. For the day 1 extinction stage, an ANOVA including the within-subjects factors *Contingency* (CS+, CS-) x *Hemisphere* (left, right) x *Electrode* (T7/8, TP7/8, TP9/10, P7/8, PO9/10) and the between-subjects factor *Group* (yohimbine, sulpiride, placebo) was computed. The ANOVA for the day 2 fear/extinction recall phase included the additional within-subjects factor *Extinction Status* (E = extinguished, N = nonextinguished).

LPP (Late Positive Potential). In the literature, ERPs for LPP analyses are most frequently referenced to the mastoids, which allows emotion-related LPP modulations to be better highlighted [48]. Thus, the EEG was referenced against the average of TP9 and TP10 (mastoids) to analyze LPP responses. Next, we computed ERPs covering 1,000 ms time-locked to the CS onsets. ERPs were baseline-corrected (200 ms pre-stimulus) and averaged across trials of each CS type. The aggregate grand average ERP [45] revealed a sustained positive deflection starting at around 400 ms after CS onset at parieto-occipital channels P1, Pz, P2, PO3, POz, PO4, O1, Oz, and O2. A robust positivity was visible from 400 to 800 ms (Supplementary Figure S1B), so we calculated the mean voltage during this time window. To analyze LPP amplitudes during day 1 extinction, we performed a *Contingency* (CS+, CS-) x *Electrode* (P1, Pz, P2, PO3, POz, PO4, O1, Oz, and O2) x *Group* (yohimbine, sulpiride, placebo) ANOVA. For the analysis of day 2 fear/extinction recall, we included the additional factor *Extinction Status* (E = extinguished, N = nonextinguished).

G Statistical Analyses

All statistical tests were performed using SPSS 24 for Windows (IBM, Armonk, NY/USA), and $p \leq .05$ (two-sided) was required to reach significance. Each experimental phase (day 1 acquisition, day 1 extinction, and day 2 recall test) was analyzed separately. Significant effects of mixed-model ANOVAs (including the between-subjects factor *Group* and several within-subjects factors, as described in the *Materials and Methods* section of the main text) were further analyzed using follow-up ANOVAs and *t*-tests within groups. The Greenhouse-Geisser [49] adjustment was used to correct for violations of sphericity.



Supplementary Figure S1. The topography (voltage maps, left panels) and waveform (right panels) of the aggregate grand average ERP (collapsed across trials of all CS types, across all experimental groups, and across day 1 extinction and day 2 recall) during the N170 and LPP periods. (A) The CSs evoked a distinct negativity at left (T7, TP7, TP9, P7, and PO9) and right (T8, TP8, TP10, P8, and PO10) occipito-temporal electrodes from 145 to 185 ms after CS onset (N170 period). The aforementioned channels were included in the ANOVA on N170 amplitudes; they are shown as white dots in the voltage map (left panel). To illustrate the ERP waveform, these channels were averaged (right panel). For N170 analyses, EEG data were referenced against Cz (gray dot in voltage map). (B) The CSs were associated with a sustained positivity from 400 to 800 ms after CS onset at parieto-occipital channels P1, Pz, P2, PO3, POz, PO4, O1, Oz, and O2. These channels were included in the ANOVA on LPP amplitudes. They are shown as white dots in the voltage map (left panel) and were averaged to display the ERP waveform (right panel). For LPP analyses, EEG data were referenced against the average of TP9 and TP10 (mastoids, gray dots in voltage map). Gray-shaded areas indicate time windows for statistical analyses. "L" = left hemisphere, "R" = right hemisphere.

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5.5 Supplementary Material for Manuscript 5

Panitz, C., **Sperl, M. F. J.**, Hennig, J., Klucken, T., Hermann, C., & Mueller, E. M. (2018). Fearfulness, neuroticism/anxiety, and COMT Val158Met in long-term fear conditioning and extinction. *Neurobiology of Learning and Memory*, *155*, 7–20. https://doi.org/10.1016/j.nlm.2018.06.001

A *COMT* Val158Met and Personality Scales

Trend for differences in Harmavoidance between genotype groups, no significant differences in any other scale, see Supplementary Tables S1 and S2.

Supplementary Table S1. ANOVA *F* values, *p* values and effect sizes (η_p^2) for comparison of genotype groups, dependent variables: personality scales.

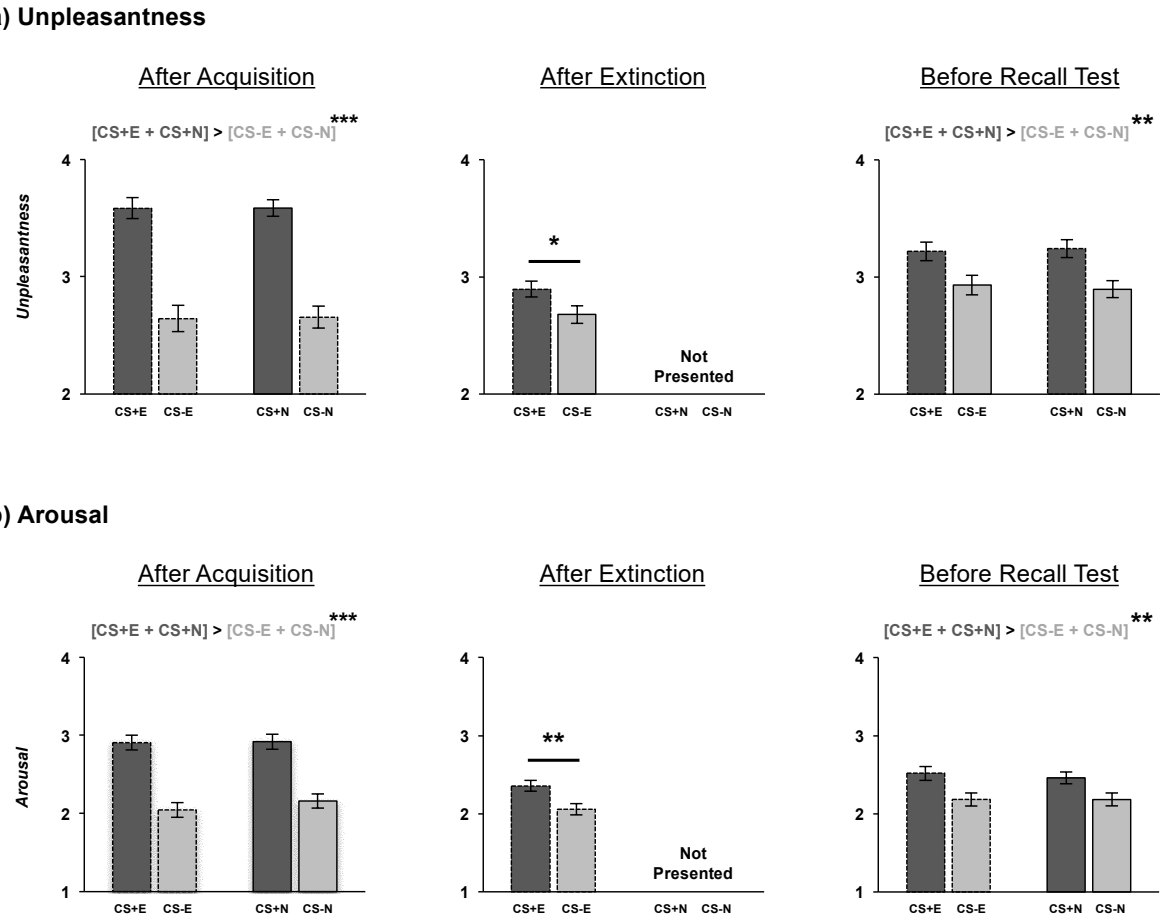
	$F(2, 84)$	p	η_p^2
MPQ – Harm Avoidance	2.64	.077	.059
ZKPQ – Sensation Seeking	0.37	.694	.009
NEO-FFI – Neuroticism	0.14	.870	.003
ZKPQ – Neuroticism/Anxiety	0.43	.653	.010
MPQ – Stress Reaction	0.85	.431	.020
BIS	0.98	.382	.023
STAI – Trait	0.40	.673	.009
ZKPQ – Activity	1.83	.166	.042
MPQ – Social Potency	0.92	.404	.021
BAS	0.17	.845	.004

Supplementary Table S2. Bivariate Correlations Between Questionnaire Scales and Conditioned Responses

	Fearfulness		*Neuroticism/Anxiety*					*Agentic Extraversion*		
	HA	*SS*	*N*	*NAnx*	*SR*	*BIS*	*STAI-T*	*Act*	*SP*	*BAS*
Unpleasantness										
ACQ	.234*	-.023	-.029	-.046	-.046	.143	-.064	-.068	-.076	.082
EXT	.117	-.040	.011	-.075	.015	.157	-.046	.011	-.070	.145
Recall (CONT)	.144	-.114	.029	.062	.025	.156	.018	-.141	-.075	-.046
Recall (CONT x EXT)	.122	-.034	-.208	-.005	-.106	-.150	-.022	.033	-.010	-.315**
Arousal										
ACQ	.126	-.053	-.066	-.092	-.147	-.070	-.067	.120	.064	-.160
EXT	-.051	-.081	.016	.022	-.052	-.062	.093	.122	.045	-.213*
Recall (CONT)	.099	-.061	.006	-.005	-.068	-.105	.019	.108	.026	-.203
Recall (CONT x EXT)	.074	-.095	.127	.071	.061	.223*	.016	-.314**	-.256*	.145
Fear Bradycardia										
ACQ	.225*	.090	-.036	-.022	-.023	.056	-.034	.024	.052	.144
EXT	.168	-.099	.084	.107	.136	.021	.141	-.017	.052	-.027
Recall (CONT)	.002	.030	.083	.022	.076	.086	.200	.033	.018	.050
Recall (CONT x EXT)	-.067	-.022	-.068	-.120	-.135	.004	-.063	-.101	-.165	-.039
SCR										
ACQ	.130	.011	.071	.068	-.001	.016	.059	-.116	-.148	.097
EXT	.125	-.093	.211*	.157	.156	.155	.150	-.038	-.114	.072
Recall (CONT)	.147	-.112	.036	.021	-.035	.086	.051	-.082	-.136	-.031
Recall (CONT x EXT)	-.099	.108	-.063	-.127	-.108	-.015	-.094	.089	.064	.066
LPP										
EXT	.071	.110	-.116	-.120	-.118	-.033	-.056	-.096	.006	-.018
Recall (CONT)	.124	.047	-.160	-.122	-.135	--.201	-.158	-.102	.090	-.023
Recall (CONT x EXT)	.087	.016	-.141	-.154	-.180	-.107	-.141	.143	.096	.143

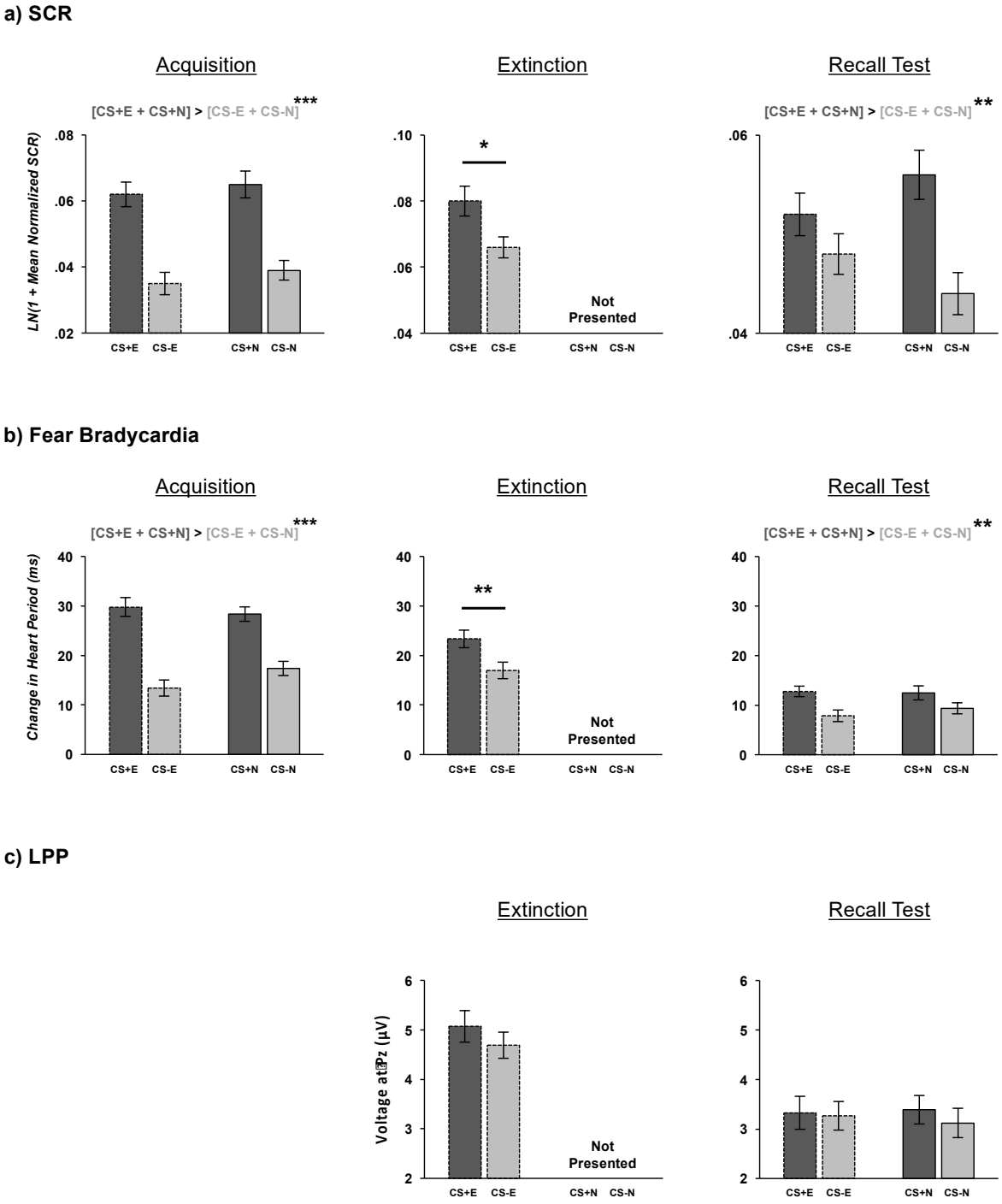
Note: $^{*}p < .05$, $^{**}p < .01$, two-sided. ACQ = main effect of Contingency during acquisition; EXT = main effect of Contingency during extinction; Recall (CONT) = contrast for main effect of Contingency during recall; Recall (CONT x EXT) = contrast for Contingency x Extinction interaction during recall; SCR = skin conductance response; LPP = Late Positive Potential; HA = MPQ Harmavoidance; SS = ZKPQ Sensation Seeking; N = NEO-FFI Neuroticism; NAnx = ZKPQ-Neuroticism/Anxiety; SR = MPQ Stress Reaction; BIS = Carver & White BIS; STAI-T = STAI-Trait; Act = ZKPQ Activity; SP = MPQ Social Potency; BAS = Carver & White BAS.

B Unpleasantness/Arousal Ratings for all Phases, Across *COMT* Val158Met Groups



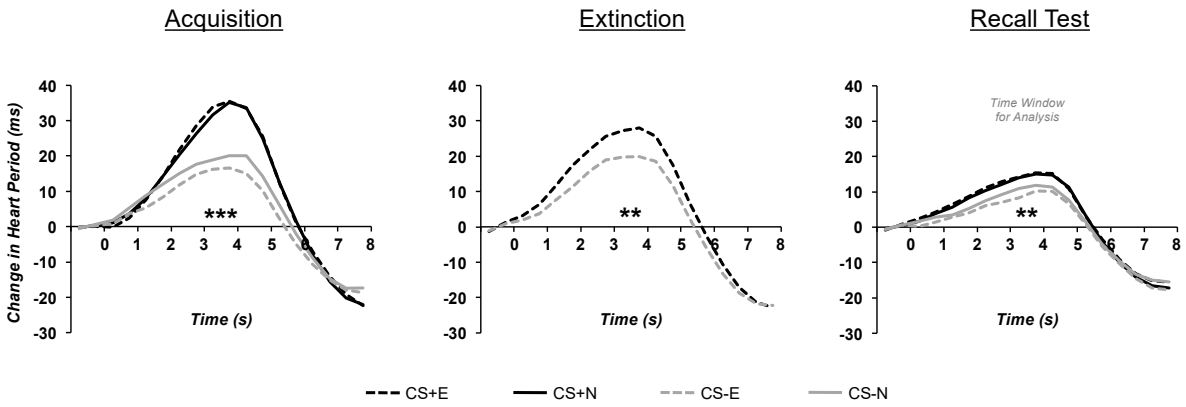
Supplementary Figure S1. Means and *SEM* for affective ratings to the different CS across genotype groups after Day 1 acquisition, after Day 1 extinction, and before Day 2 recall test phases. CS+E = extinguished CS+, CS+N = non-extinguished CS+, CS-E = CS- presented during extinction phase, CS-N = CS- not presented during extinction phase. Main effects of contingency: ***p < .001, **p < .01, *p < .05. Error bars indicate *SEM* based on within-subject variance. (a) unpleasantness ratings. (b) arousal ratings.

C Physiological Conditioned Responses for all Phases, Across *COMT* Val158Met Groups



Supplementary Figure S2. Means and *SEM* for physiological responses to the different CS across genotype groups during Day 1 acquisition, Day 1 extinction, and Day 2 recall test phases. CS+E = extinguished CS+, CS+N = non-extinguished CS+, CS-E = CS- presented during extinction phase, CS-N = CS- not presented during extinction phase. Main effects of contingency: ***p < .001, **p < .01, *p < .05. Error bars indicate *SEM* based on within-subject variance. (a) LN-transformed mean normalized SCR. (b) Change in HP relative to baseline (fear bradycardia). (c) ERP at electrode Pz.

D CS-Evoked Heart Period Changes for all Phases, Across *COMT* Val158Met Groups



Supplementary Figure S3. Change in HP relative to baseline across genotype groups during Day 1 acquisition, Day 1 extinction and Day 2 extinction recall, respectively. Mean magnitude within the shaded area was used for statistical analyses of fear bradycardia. CS+E = extinguished CS+, CS+N = non-extinguished CS+, CS-E = CS- presented during extinction phase, CS-N = CS- not presented during extinction phase. Main effects of Contingency: ***$p < .001$, **$p < .01$.

E ANCOVAs including Contingency, Extinction, Genotype (Independent Factors) and Personality Compound Scores (Covariates)

Supplementary Table S3. ANCOVA effects for unpleasantness ratings after Day 1 acquisition.

	F	df_{effect}	df_{error}	p	η_p^2
Contingency (Cont) ^{A}	44.68	1	81	< .001***	.356
Extinction (Ext)	0.00	1	81	.981	.000
Genotype (Gen)	0.57	2	81	.570	.014
Fearfulness (Fear)	3.58	1	81	.062	.042
Neuroticism/Anxiety (N/Anx)	0.04	1	81	.850	.000
Agentic Extraversion (agE)	0.31	1	81	.581	.004
Cont x Ext	0.00	1	81	.954	.000
Cont x Gen	0.73	2	81	.486	.018
Cont x Fear ^{R}	6.37	1	81	.014*	.073
Cont x N/Anx	0.00	1	81	.992	.000
Cont x agE	0.00	1	81	.984	.000
Ext x Gen	2.06	2	81	.135	.048
Ext x Fear	0.08	1	81	.783	.001
Ext x N/Anx	6.00	1	81	.016*	.069
Ext x agE	0.49	1	81	.488	.006

Cont x Ext x Gen	0.11	2	81	.899	.003
Cont x Ext x Fear	1.04	1	81	.312	.013
Cont x Ext x N/Anx	0.75	1	81	.388	.009
Cont x Ext x agE	0.28	1	81	.598	.003

Note. * $p < .05$, *** $p < .001$, ^{A} significant effect in ANOVA, ^{R} significant effect in regression analysis.

Supplementary Table S4. ANCOVA effects for arousal ratings after Day 1 acquisition.

	F	df_{effect}	df_{error}	p	η_p^2
Contingency (Cont)^{A}	28.38	1	81	< .001***	.259
Extinction (Ext)	0.77	1	81	.381	.009
Genotype (Gen)	0.21	2	81	.815	.005
Fearfulness (Fear)	3.14	1	81	.080	.037
Neuroticism/Anxiety (N/Anx)	1.19	1	81	.279	.014
Agentic Extraversion (agE)	0.10	1	81	.750	.001
Cont x Ext	0.38	1	81	.538	.005
Cont x Gen	0.07	2	81	.930	.002
Cont x Fear	1.18	1	81	.281	.014
Cont x N/Anx	0.70	1	81	.405	.009
Cont x agE	0.00	1	81	.986	.000
Ext x Gen	1.11	2	81	.336	.027
Ext x Fear	0.10	1	81	.751	.001
Ext x N/Anx	1.92	1	81	.169	.023
Ext x agE	0.70	1	81	.406	.009
Cont x Ext x Gen	0.77	2	81	.467	.019
Cont x Ext x Fear	0.13	1	81	.716	.002
Cont x Ext x N/Anx	0.93	1	81	.337	.011
Cont x Ext x agE	0.25	1	81	.620	.003

Note. *** $p < .001$, ^{A} significant effect in ANOVA.

Supplementary Table S5. ANCOVA effects for fear bradycardia during Day 1 acquisition.

	F	df_{effect}	df_{error}	p	η_p^2
Contingency (Cont)^{A}	36.25	1	81	< .001***	.309
Extinction (Ext)	0.69	1	81	.410	.008
Genotype (Gen)	0.43	2	81	.650	.011
Fearfulness (Fear)	3.51	1	81	.065	.042

Neuroticism/Anxiety (N/Anx)	0.03	1	81	.868	.000
Agentic Extraversion (agE)	2.01	1	81	.160	.024
Cont x Ext	2.13	1	81	.149	.026
Cont x Gen	1.90	2	81	.156	.045
Cont x Fear^{R}	9.83	1	81	.002**	.108
Cont x N/Anx	0.05	1	81	.832	.001
Cont x agE	1.60	1	81	.209	.019
Ext x Gen^{A}	2.90	2	81	.061	.067
Ext x Fear	0.79	1	81	.378	.010
Ext x N/Anx	0.04	1	81	.847	.000
Ext x agE	2.867	1	81	.094	.034
Cont x Ext x Gen	0.61	2	81	.547	.015
Cont x Ext x Fear	2.05	1	81	.156	.025
Cont x Ext x N/Anx	0.16	1	81	.687	.002
Cont x Ext x agE	0.29	1	81	.595	.004

Note. ** $p < .01$, *** $p < .001$, ^{A} significant effect in ANOVA, ^{R} significant effect in regression analysis.

Supplementary Table S6. ANCOVA effects for SCR during Day 1 acquisition.

	F	df_{effect}	df_{error}	p	η_p^2
Contingency (Cont)^{A}	27.71	1	81	< .001***	.255
Extinction (Ext)	1.11	1	81	.294	.014
Genotype (Gen)	1.29	2	81	.281	.031
Fearfulness (Fear)	1.07	1	81	.305	.013
Neuroticism/Anxiety (N/Anx)	0.65	1	81	.422	.008
Agentic Extraversion (agE)	2.15	1	81	.146	.026
Cont x Ext	0.02	1	81	.883	.000
Cont x Gen	0.50	2	81	.606	.012
Cont x Fear	2.40	1	81	.126	.029
Cont x N/Anx	0.17	1	81	.679	.002
Cont x agE	0.12	1	81	.734	.001
Ext x Gen	2.90	2	81	.061	.067
Ext x Fear	1.11	1	81	.294	.014
Ext x N/Anx	0.41	1	81	.524	.005
Ext x agE	0.10	1	81	.746	.001
Cont x Ext x Gen	0.46	2	81	.631	.011
Cont x Ext x Fear	.873	1	81	.353	.011

Cont x Ext x N/Anx	0.50	1	81	.484	.006
Cont x Ext x agE	0.87	1	81	.355	.011

Note. *** $p < .001$, [A] significant effect in ANOVA.

Supplementary Table S7. ANCOVA effects for unpleasantness ratings after Day 1 extinction.

	F	df_{effect}	df_{error}	p	η_p^2
Contingency (Cont)[A]	4.14	1	81	.045*	.049
Genotype (Gen)	0.40	2	81	.669	.010
Fearfulness (Fear)	3.79	1	81	.055	.045
Neuroticism/Anxiety (N/Anx)	0.24	1	81	.629	.003
Agentic Extraversion (agE)	0.58	1	81	.449	.007
Cont x Gen	1.12	2	81	.330	.027
Cont x Fear	1.06	1	81	.307	.013
Cont x N/Anx	0.16	1	81	.695	.002
Cont x agE	0.27	1	81	.606	.003

Note. * $p < .05$, [A] significant effect in the initial ANOVA. Note that there was no Extinction factor in this ANCOVA model as only two out of four CS (CS+E, CS-E) were presented during extinction phase.

Supplementary Table S8. ANCOVA effects for arousal ratings after Day 1 extinction.

	F	df_{effect}	df_{error}	p	η_p^2
Contingency (Cont)[A]	7.03	1	81	.010*	.080
Genotype (Gen)	0.76	2	81	.472	.018
Fearfulness (Fear)	0.74	1	81	.391	.009
Neuroticism/Anxiety (N/Anx)	0.29	1	81	.591	.004
Agentic Extraversion (agE)	0.20	1	81	.472	.018
Cont x Gen	0.06	2	81	.941	.001
Cont x Fear	0.64	1	81	.427	.008
Cont x N/Anx	0.00	1	81	.985	.000
Cont x agE	0.04	1	81	.851	.000

Note. * $p < .05$, [A] significant effect in ANOVA. Note that there was no Extinction factor in this ANCOVA model as only two out of four CS (CS+E, CS-E) were presented during extinction phase.

Supplementary Table S9. ANCOVA effects for fear bradycardia during Day 1 extinction.

	F	df_{effect}	df_{error}	p	η_p^2
Contingency (Cont)[A]	10.45	1	81	.002**	.114
Genotype (Gen)	1.29	2	81	.282	.031

Fearfulness (Fear)	1.74	1	81	.192	.021
Neuroticism/Anxiety (N/Anx)	2.98	1	81	.088	.035
Agentic Extraversion (agE)	0.03	1	81	.861	.000
Cont x Gen	0.99	2	81	.375	.024
Cont x Fear	1.18	1	81	.281	.014
Cont x N/Anx	0.73	1	81	.394	.009
Cont x agE	0.02	1	81	.889	.000

Note. ** $p < .01$, [A] significant effect in ANOVA. Note that there was no Extinction factor in this ANCOVA model as only two out of four CS (CS+E, CS-E) were presented during extinction phase.

Supplementary Table S10. ANCOVA effects for SCR during Day 1 extinction.

	F	df_{effect}	df_{error}	p	η_p^2
Contingency (Cont)[A]	5.77	1	81	.019*	.067
Genotype (Gen)	2.93	2	81	.059	.067
Fearfulness (Fear)	8.77	1	81	.004**	.098
Neuroticism/Anxiety (N/Anx)	0.03	1	81	.862	.000
Agentic Extraversion (agE)	5.30	1	81	.024*	.061
Cont x Gen	2.55	2	81	.084	.059
Cont x Fear	0.73	1	81	.395	.009
Cont x N/Anx	2.25	1	81	.138	.027
Cont x agE	0.00	1	81	.996	.000

Note. * $p < .05$, ** $p < .01$, [A] significant effect in ANOVA. Note that there was no Extinction factor in this ANCOVA model as only two out of four CS (CS+E, CS-E) were presented during extinction phase.

Supplementary Table S11. ANCOVA effects for LPP during Day 1 extinction.

	F	df_{effect}	df_{error}	p	η_p^2
Contingency (Cont)[A]	3.33	1	81	.072	.040
Genotype (Gen)	0.33	2	81	.720	.008
Fearfulness (Fear)	3.90	1	81	.052	.046
Neuroticism/Anxiety (N/Anx)	1.40	1	81	.241	.017
Agentic Extraversion (agE)	1.57	1	81	.214	.019
Cont x Gen	2.86	2	81	.063	.066
Cont x Fear	2.97	1	81	.089	.035
Cont x N/Anx	0.84	1	81	.364	.010
Cont x agE	0.08	1	81	.777	.001

Note. [A] significant effect in ANOVA for one-sided test. Note that there was no Extinction factor in this ANCOVA model as only two out of four CS (CS+E, CS-E) were presented during extinction phase.

Supplementary Table S12. ANCOVA effects for unpleasantness ratings before Day 2 recall.

	F	df_{effect}	df_{error}	p	η_p^2
Contingency (Cont) ^{A}	9.41	1	81	.003**	.104
Extinction (Ext)	0.02	1	81	.896	.000
Genotype (Gen)	0.87	2	81	.423	.021
Fearfulness (Fear)	0.02	1	81	.886	.000
Neuroticism/Anxiety (N/Anx)	0.37	1	81	.544	.005
Agentic Extraversion (agE)	2.65	1	81	.107	.032
Cont x Ext	0.10	1	81	.748	.001
Cont x Gen	0.97	2	81	.383	.023
Cont x Fear	1.05	1	81	.309	.013
Cont x N/Anx	0.30	1	81	.587	.004
Cont x agE	0.57	1	81	.454	.007
Ext x Gen	1.92	2	81	.153	.045
Ext x Fear	0.42	1	81	.519	.005
Ext x N/Anx	2.02	1	81	.159	.024
Ext x agE	0.21	1	81	.651	.003
Cont x Ext x Gen	0.17	2	81	.847	.004
Cont x Ext x Fear	1.28	1	81	.260	.016
Cont x Ext x N/Anx	1.74	1	81	.191	.021
Cont x Ext x agE	1.82	1	81	.182	.022

Note. ** $p < .01$, ^{A} significant effect in ANOVA.

Supplementary Table S13. ANCOVA effects for arousal ratings before Day 2 recall.

	F	df_{effect}	df_{error}	p	η_p^2
Contingency (Cont) ^{A}	7.97	1	81	.006**	.090
Extinction (Ext)	0.09	1	81	.771	.001
Genotype (Gen)	1.45	2	81	.240	.035
Fearfulness (Fear)	0.15	1	81	.696	.002
Neuroticism/Anxiety (N/Anx)	0.03	1	81	.868	.000
Agentic Extraversion (agE)	0.84	1	81	.361	.010
Cont x Ext	0.11	1	81	.744	.001
Cont x Gen	0.68	2	81	.507	.017
Cont x Fear	0.15	1	81	.705	.002
Cont x N/Anx	0.29	1	81	.589	.004
Cont x agE	0.31	1	81	.579	.004

Ext x Gen	0.96	2	81	.387	.023
Ext x Fear	2.14	1	81	.148	.026
Ext x N/Anx	1.95	1	81	.167	.023
Ext x agE	0.24	1	81	.624	.003
Cont x Ext x Gen	0.38	2	81	.686	.009
Cont x Ext x Fear	0.01	1	81	.933	.000
Cont x Ext x N/Anx	0.34	1	81	.560	.004
Cont x Ext x agE	2.57	1	81	.113	.031

Note. ** $p < .01$, [A] significant effect in ANOVA.

Supplementary Table S14. ANCOVA effects for fear bradycardia during Day 2 recall.

	F	df_{effect}	df_{error}	p	η_p^2
Contingency (Cont)[A]	7.24	1	81	.009**	.082
Extinction (Ext)	0.23	1	81	.632	.003
Genotype (Gen)	0.56	2	81	.572	.014
Fearfulness (Fear)	2.13	1	81	.149	.026
Neuroticism/Anxiety (N/Anx)	0.12	1	81	.733	.001
Agentic Extraversion (agE)	0.02	1	81	.895	.000
Cont x Ext	0.91	1	81	.343	.011
Cont x Gen	0.77	2	81	.467	.019
Cont x Fear	0.04	1	81	.846	.000
Cont x N/Anx	0.95	1	81	.333	.012
Cont x agE	0.19	1	81	.664	.002
Ext x Gen	0.98	2	81	.379	.024
Ext x Fear	1.00	1	81	.319	.012
Ext x N/Anx	0.47	1	81	.497	.006
Ext x agE	0.59	1	81	.443	.007
Cont x Ext x Gen	1.15	2	81	.321	.028
Cont x Ext x Fear	1.24	1	81	.270	.015
Cont x Ext x N/Anx	1.35	1	81	.248	.016
Cont x Ext x agE	2.71	1	81	.103	.032

Note. ** $p < .01$, [A] significant effect in ANOVA.

Supplementary Table S15. ANCOVA effects for SCR during Day 2 Recall.

	F	df_{effect}	df_{error}	p	η_p^2
Contingency (Cont)[A2]	7.80	1	81	.007**	.088

Extinction (Ext)	0.05	1	81	.822	.001
Genotype (Gen)	1.70	2	81	.188	.040
Fearfulness (Fear)	5.62	1	81	.020*	.065
Neuroticism/Anxiety (N/Anx)	1.01	1	81	.318	.012
Agentic Extraversion (agE)	0.74	1	81	.392	.009
Cont x Ext^{A1}	3.52	1	81	.064	.042
Cont x Gen	0.31	2	81	.735	.008
Cont x Fear	1.22	1	81	.273	.015
Cont x N/Anx	0.01	1	81	.923	.923
Cont x agE	0.69	1	81	.410	.008
Ext x Gen	0.58	2	81	.562	.014
Ext x Fear	0.61	1	81	.436	.008
Ext x N/Anx	1.29	1	81	.259	.016
Ext x agE	0.09	1	81	.768	.001
Cont x Ext x Gen	1.99	2	81	.143	.047
Cont x Ext x Fear	0.07	1	81	.798	.001
Cont x Ext x N/Anx	0.13	1	81	.715	.002
Cont x Ext x agE	0.90	1	81	.347	.011

Note. * $p < .05$, ** $p < .01$, ^{A2} significant effect in ANOVA for two-sided test, ^{A1} significant effect in ANOVA for one-sided test.

Supplementary Table S16. ANCOVA effects for LPP during Day 2 Recall.

	F	df_{effect}	df_{error}	p	η_p^2
Contingency (Cont)	0.84	1	81	.361	.010
Extinction (Ext)	0.10	1	81	.757	.001
Genotype (Gen)	0.58	2	81	.563	.014
Fearfulness (Fear)	7.39	1	81	.008**	.084
Neuroticism/Anxiety (N/Anx)	1.29	1	81	.259	.016
Agentic Extraversion (agE)	0.03	1	81	.869	.000
Cont x Ext	0.33	1	81	.570	.004
Cont x Gen	1.15	2	81	.321	.028
Cont x Fear	1.36	1	81	.247	.017
Cont x N/Anx	2.74	1	81	.102	.033
Cont x agE	0.33	1	81	.569	.004
Ext x Gen	0.31	2	81	.737	.008
Ext x Fear	0.01	1	81	.905	.000

Ext x N/Anx	0.04	1	81	.850	.000
Ext x agE	0.19	1	81	.667	.002
Cont x Ext x Gen ^{A}	2.78	2	81	.068	.064
Cont x Ext x Fear	0.10	1	81	.757	.001
Cont x Ext x N/Anx	2.09	1	81	.152	.025
Cont x Ext x agE	0.71	1	81	.402	.009

Note. ** $p < .01$, ^{A} significant effect in ANOVA.

F Neuroticism/Anxiety and Day-1/Day-2 Stability of Extinguished Fear Bradycardia

Modulation of Day-1/Day-2 stability by neuroticism/anxiety scales, see Supplementary Table S17.

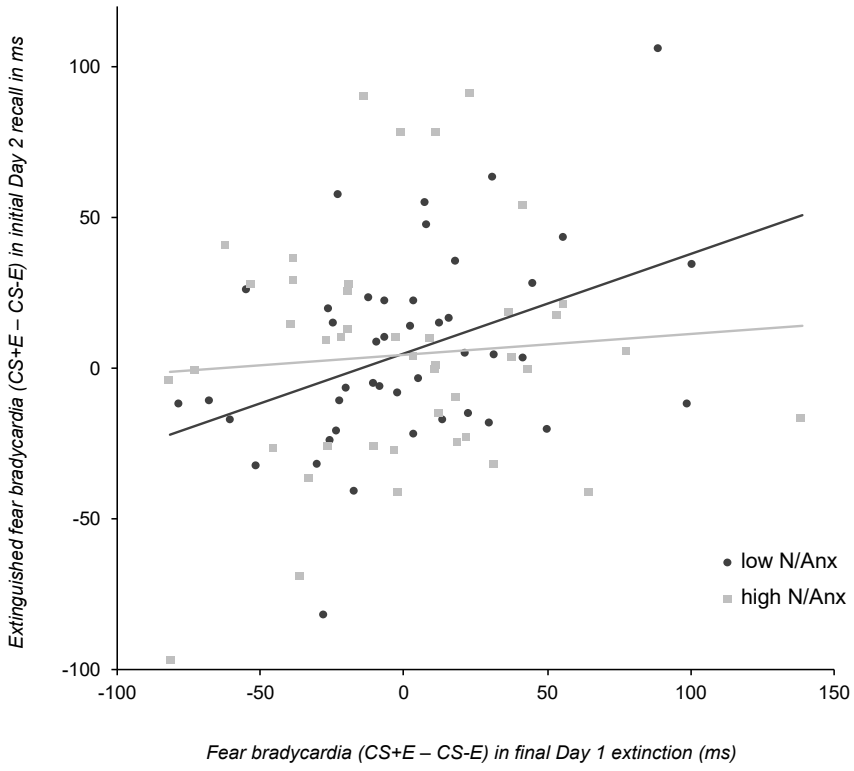
Supplementary Table S17. Multiple regression beta weights and *p*-values for interaction between final Day 1 extinction fear bradycardia (last ten trials) and neuroticism/anxiety scales in the prediction of initial Day 2 extinguished fear bradycardia (first ten trials).

	beta	*p*-value
Day 1 Fear Bradycardia	**.223***	**.030**
ZKPQ – Neuroticism/Anxiety	.065	.529
Interaction	**-.340****	**.001**
Day 1 Fear Bradycardia	.182	.076
MPQ – Stress Reaction	.105	.306
Interaction	**-.340****	**.001**
Day 1 Fear Bradycardia	**.261***	**.012**
BIS	-.018	.867
Interaction	**-.318****	**.003**
Day 1 Fear Bradycardia	.209	.051

STAI – Trait	.020	.853
Interaction	-.186	.088
Day 1 Fear Bradycardia	.209	.051
NEO-FFI – Neuroticism	-.085	.435
Interaction	-.130	.236

Note. * $p < .05$ ** $p < .01$. This table depicts results from five different regression analyses with the predictors Day 1 Fear Bradycardia, Personality Questionnaire, and the interaction term, respectively. This is unlike the analysis in the main manuscript, where all compound scores and interaction terms were entered simultaneously into the regression analysis.

Modulation of Day-1/Day-2 stability by neuroticism/anxiety compound score, see Supplementary Figure S4.



Supplementary Figure S4. Relationship between fear bradycardia in final Day 1 extinction and extinguished fear bradycardia in initial Day 2 recall (CS+E – CS-E) separate for low vs. high neuroticism/anxiety scores as determined via median split. Please note that the median split was only used for this figure, the regression analysis in the manuscript was conducted using the continuous neuroticism/anxiety scores.

G Power Analyses

Supplementary Table S18. Power analyses for significant genotype and personality effects of interest.

	r_p^2	*1-β*	$N_{two\text{-}sided}$	$N_{one\text{-}sided}$
Fearfulness – unpleasantness (Day 1)	.060	.644	125	99
Fearfulness – fear bradycardia (Day 1)	.082	.787	90	71
Neuroticism/Anxiety – Day-1/Day-2 stability of fear bradycardia	.083	.792	89	69
	η_p^2	*1-β*	$N_{two\text{-}sided}$	
Genotype x Contingency x Extinction interaction in initial Day 2 fear bradycardia	.100	.771	93	
Genotype x Contingency x Extinction interaction in Day 2 late positive potential	.075	.626	126	
Genotype – Day-1/Day-2 stability of fear bradycardia	.081	.647	117	

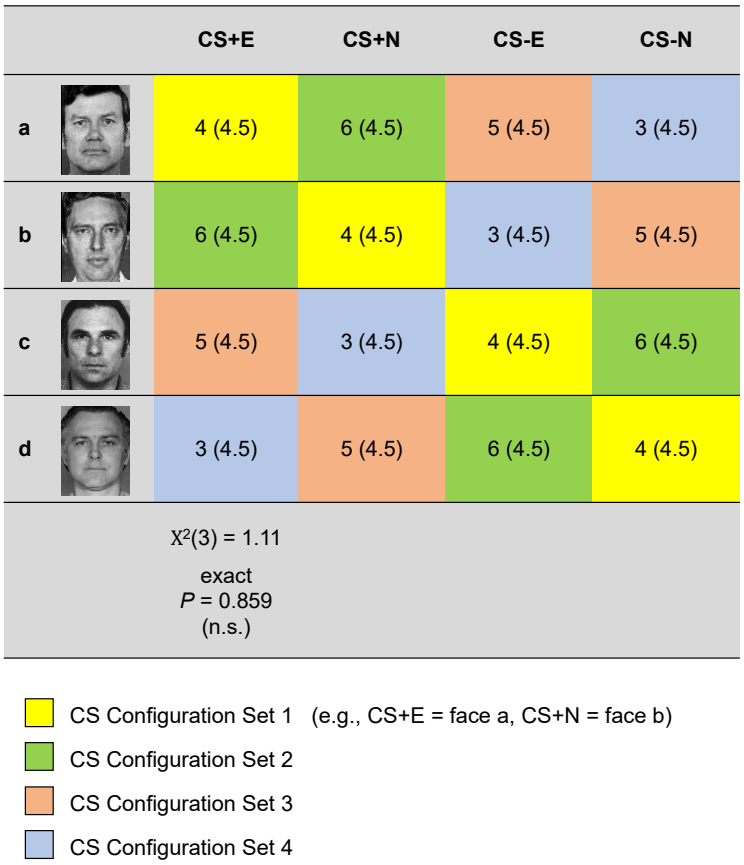
Note. r_p^2 = squared partial correlation of predictor and criterion in multiple regression model, η_p^2 partial eta square for AN(C)OVA effect, 1-β = statistical power (α = .05, two-sided test), $N_{two\text{-}sided}$ = necessary sample size to replicate effect with a two-sided test (α = .05, 1-β = .80), $N_{two\text{-}sided}$ = necessary sample size to replicate effect with a two-sided test (α = .05, 1-β = .80).

5.6 Supplementary Material for Manuscript 6

Sperl, M. F. J., Panitz, C., Rosso, I. M., Dillon, D. G., Kumar, P., Hermann, A., Whitton, A. E., Hermann, C., Pizzagalli, D. A., & Mueller, E. M. (2019). Fear extinction recall modulates human frontomedial theta and amygdala activity. *Cerebral Cortex*, *29*, 701–715. https://doi.org/10.1093/cercor/bhx353

A Counterbalanced Assignment of Face Stimuli to CS types

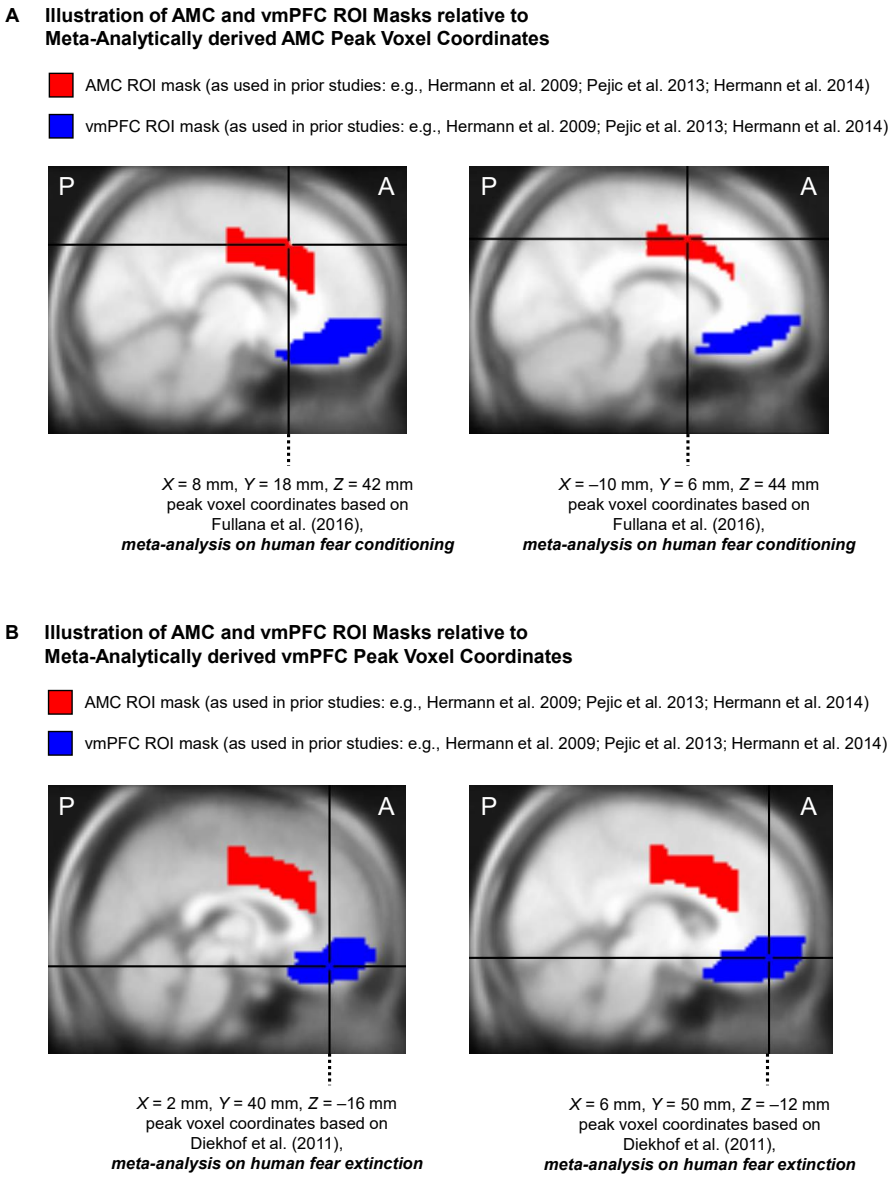
Four different black-and-white male faces with a neutral expression (Ekman and Friesen 1976) constituted the CSs. The assignment of face stimuli to CS+E, CS+N, CS-E, and CS-N was permutated in a counterbalanced fashion. Prior to the experiment, four different CS configuration sets were designed in a fashion which ensured counterbalancing of assignment of face stimuli. Afterwards, each subject was randomly assigned to a specific CS configuration set. After the exclusion of three subjects with complete absence of explicit CS-US contingency awareness, reasonable counterbalancing was still achieved (see Supplementary Figure S1). Thus, no specific CS type was disproportionally linked to a specific face stimulus, $X^2(3) = 1.11$, exact $P = 0.859$.



Supplementary Figure S1. Counterbalanced assignment of face stimuli to CS+E, CS+N, CS-E, and CS-N. The figure depicts the observed count of each possible CS-face combination. The count which would be expected under the assumption of no association between CS type and face stimuli is indicated in brackets.

B Additional Information and Visualization of AMC/vmPFC ROI Masks

The masks for AMC and vmPFC were created in the MARINA software package (Walter et al. 2003) according to the parcellation of Tzourio-Mazoyer et al. (2002), and were identical to the ones used in previous studies (e.g., Hermann et al. 2009; Pejic et al. 2013; Hermann, Keck, Stark 2014). The AMC mask consists of the bilateral cingulate and paracingulate gyri and ranges from $y = 32$ to $y = -18$ (MNI coordinates) with regard to the AC-PC line (Supplementary Figure S2A). This mask includes the two peak coordinates reported in a recently published meta-analysis on fear conditioning (Fullana et al. 2016). The vmPFC mask consists of the bilateral medial orbital area of the frontal cortex and the gyrus rectus (Supplementary Figure S2B), including the two peak voxels identified by a meta-analysis on fear extinction (Diekhof et al. 2011).



Supplementary Figure S2. Illustration of AMC (in red) and vmPFC (in blue) ROI masks, which have been created according to the parcellation of Tzourio-Mazoyer et al. (2002). Identical masks have been used in previous studies (e.g., Hermann et al. 2009; Pejic et al. 2013; Hermann, Keck, Stark 2014). (A) The AMC and (B) vmPFC masks include meta-analytically derived peak coordinates (Diekhof et al. 2011; Fullana et al. 2016). A = anterior, P = posterior.

C Control EEG Analysis Without ICA Eye Blink/Movement Correction (Day 2 Recall)

Whereas blinks and eye movements do not generate considerable electrophysiological activity outside the delta and theta range, potential contamination of theta activity by ocular artifacts is a major issue (Gasser, Sroka, Möcks 1985; Hagemann and Naumann 2001). Specifically, Hagemann and Naumann (2001) reported greater power density within the theta band at all electrode sites for segments with severe ocular artifacts. As we applied ICA to remove artifacts from eye blinks and movements, it was important to perform additional control analyses to ensure theta effects described in the main text were not partially be driven by differential eye movements across conditions. In fact, ocular artifacts are often separated into different ICA components and successful identification of all components may be challenging. Therefore, we performed an additional EEG analysis of the original data. Instead of using ICA-based eye blink/movement correction, these analyses considered only artifact-free epochs. Importantly, confirming the validity of our findings, results for the *Contingency* x *Extinction* interaction revealed a comparable topography and confirmed the sensitivity of frontomedial theta oscillations for fear and extinction recall (Supplementary Figure S3A). At frontal-midline channel Fz, a trend for a *Contingency* x *Extinction* interaction, $F(1,17) = 4.21$, $P = 0.056$, showed that differential (CS+ versus CS-) frontomedial theta power was reduced for extinguished versus nonextinguished stimuli. Overall, *F*/*t*-values were descriptively lower when we excluded artifact epochs, compared to ICA correction. We assume that the reduced number of trials (Supplementary Figure S3B) resulted in reduced statistical power due to a lower signal-to-noise ratio (Kappenman and Luck 2010).

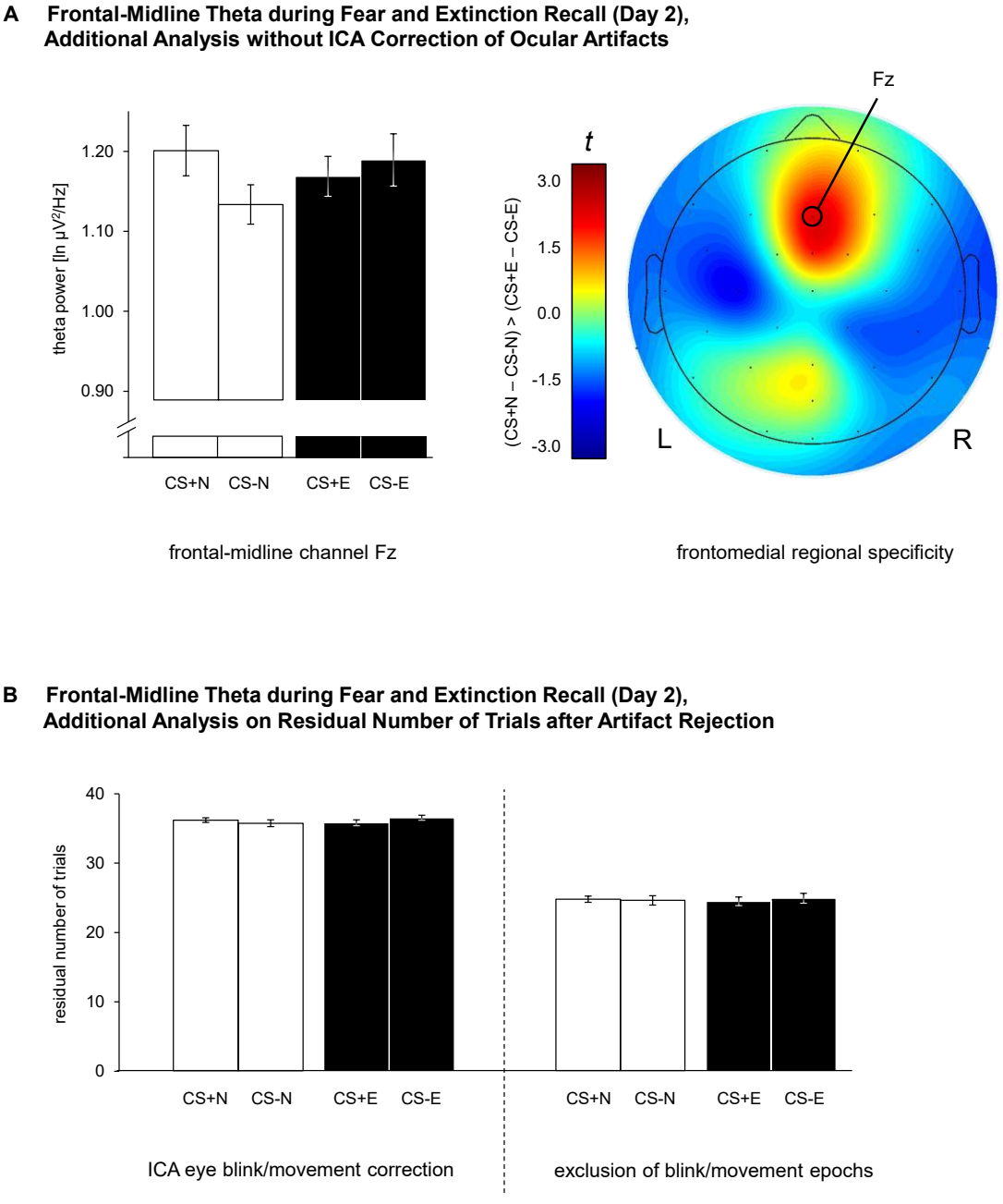
D Information on Residual Number of Trials per CS Condition After Artifact Rejection

The residual number of trials per condition after artifact rejection is shown in Supplementary Figure S3B. Information on the residual number of trials is included for both analytic strategies (i.e., with versus without ICA eye blink/movement correction). As expected, the *Contingency* x *Extinction* x *Analysis Strategy* ANOVA showed a significant main effect of *Analysis Strategy*, $F(1,17) = 41.46$, $P < 0.001$. The residual trial number was significantly lower when we limited our analysis to artifact-free epochs instead of applying ICA correction (see Supplementary Material C). There were no more significant effects involving *Contingency* or *Extinction* (*P*s $\geq$ 0.369), confirming that the residual number of trials did not differ between CS conditions.

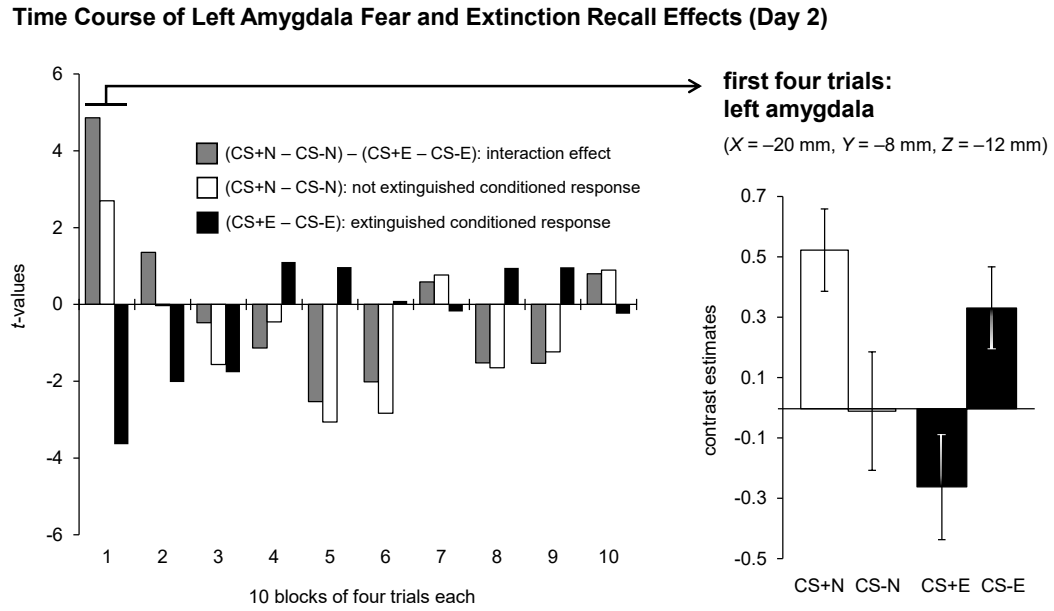
E Additional Analyses on the Time Course of Left Amygdala Effects (Day 2 Recall)

In order to account for the potentially rapid habituation of differential fear responses in the amygdala (e.g., Büchel et al. 1998b), the fMRI analysis on the Day 2 recall phase was based on a comparison between CS regressors modeling an exponentially decaying function (parametric modulation). Therefore, we explicitly tested for a *Time* x *Fear/Extinction Recall* interaction. As Büchel et al. (1998b) have pointed out, this analysis strategy tests for brain areas in which differential not extinguished conditioned responses (CS+N versus CS-N) decrease over time. At the same time, this response pattern is significantly different from differential extinguished conditioned responses (CS+E versus CS-E). This "differential adaptation" (Büchel et al. 1998b) for nonextinguished compared to extinguished differential amygdala responses is illustrated in Supplementary Figure S4. To show the time course of left amygdala effects, we followed the reviewer's suggestion and split CS regressors of the first-level GLM into ten regressors of four trials each, instead of applying an exponential modulation. Next, we extracted left amygdala contrast estimates for each CS regressor and calculated t-tests for fear (CS+N versus CS-N) and extinction (CS+E versus CS-E) recall. As expected, *t*-values for nonextinguished stimuli (i.e.,

white bars) decreased over time, whereas *t*-values for extinguished stimuli (i.e., black bars) showed an increase. Most importantly, enhanced amygdala activation for CS+N versus CS-N during early trials was reflected by positive *t*-values for nonextinguished stimuli, whereas extinguished stimuli were associated with negative *t*-values during early recall trials, indicating reduced amygdala activation for extinguished CS+E compared to CS-E (Phelps et al. 2004; Hermann et al. 2016).



Supplementary Figure S3. Additional control analyses on EEG frontomedial (Fz) theta power on Day 2. (A) In order to probe the validity of our findings, we performed an additional EEG analysis and included only artifact-free epochs, instead of ICA eye blink/movement correction. Importantly, results for the *Contingency* x *Extinction* interaction revealed a comparable topography. (B) The residual number of trials after artifact rejection was significantly lower when the EEG analysis was limited to artifact-free epochs instead of applying ICA eye blink/movement correction. Importantly, the residual number of trials was similar across CS conditions.



Supplementary Figure S4. Additional control analyses on fMRI amygdala activation on Day 2. Time course of left amygdala fear and extinction recall effects. CS regressors of the first-level GLM were split into ten regressors of four trials each, instead of applying an exponential modulation. Next, we extracted left amygdala contrast estimates for each CS regressor and calculated t-tests for fear (CS+N versus CS-N) and extinction (CS+E versus CS-E) recall. Bar graphs show the mean contrast estimates (± within-subject *SEM*, O'Brien and Cousineau, 2014) for a cluster of voxels with $P \leq 0.005$ (uncorrected) surrounding the peak voxel within the amygdala ROI.

F Control fMRI Analysis on First Four Recall Trials (Day 2)

To enhance comparability with studies of Milad and colleagues (e.g., Milad et al. 2007b; Milad et al. 2009; Milad et al. 2013; Hermann et al. 2016) and to further evaluate the validity of our findings, we performed an additional analysis for Day 2 recall which was restricted to the first four trials of each CS type. Therefore, instead of applying an exponential modulation, CS regressors of the first-level GLM were split into ten regressors of four trials each. Our main findings on amygdala activation was confirmed with both strategies (see Supplementary Table S1). In addition, a negative correlation ($P = 0.038$) of theta EEG and vmPFC BOLD modulation emerged (Supplementary Figure S5), consistent with a putative inhibitory role of the vmPFC on fear expression during early extinction recall (for a review: Milad and Quirk 2012).

G Additional Multivariate Analysis on EEG-fMRI Covariation (Day 2 Recall)

The described covariation between theta power and amygdala activation is based on covariation of FER scores, which represent a combination of fear and extinction recall. However, it remains unclear whether this correlation reflects covariation of fear-related (CS+N versus CS-N) or extinction-related (CS+E versus CS-E) effects. To address this issue, FER amygdala and theta scores were split into two scores each reflecting either the retrieval of fear (CS+N minus CS-N) or extinction memory (CS+E minus CS-E). Contrast estimates from the right amygdala cluster were extracted and *z*-standardized within individuals to estimate a quantification of amygdala effects. Next, we used multivariate regression analysis and tested whether theta modulations of fear (CS+N

minus CS-N) and extinction (CS+E minus CS-E) retrieval significantly predicted right amygdala indices of fear (CS+N minus CS-N) and extinction (CS+E minus CS-E) recall. Converging with our main findings of enhanced theta power for nonextinguished CS+N compared to CS-N, but not for extinguished CS+E compared to CS-E (see Results section), the results of this additional multivariate analysis showed that there was a significant covariation of amygdala effects with theta fear recall scores (i.e., CS+N compared to CS-N, $F(2,14) = 8.31$, $P = 0.004$), but not with theta extinction recall scores (i.e., CS+E compared to CS-E, $F(2,14) = 3.34$, $P = 0.065$). Specifically, separate follow-up univariate multiple regression analyses revealed that fear-related EEG theta modulations (CS+N minus CS-N) negatively predicted extinction-related fMRI amygdala responses (CS+E minus CS-E, $\beta = -0.74$, $P = 0.001$; see Supplementary Table S2 for statistical details).

H Sensitivity Analysis on Subject Exclusion Criterion (Contingency Awareness)

Although previous studies suggest that subjective awareness of the CS-US contingency (Boddez et al. 2013) may not represent an essential condition for successful conditioning (Knight, Nguyen, Bandettini 2006; Weike, Schupp, Hamm 2007), others have argued that conscious awareness is crucially involved in human fear conditioning (Purkis and Lipp 2001; Lovibond and Shanks 2002; Lovibond et al. 2011; Weidemann, Satkunarajah, Lovibond 2016). Given that our paradigm consisted of many acquisition trials (160 CS presentations), a complete absence of CS-US contingency is an indication that subjects did not adhere to the protocol, and an inclusion of those subjects would threaten the validity of our findings. Therefore, the main analyses excluded three subjects who rated the CS- as more likely to be followed by the US than the CS+ (see Subjects section for details). However, recent recommendations (Lonsdorf et al. 2017) propose that it should be indicated whether results remain similar when such exclusion criteria are not applied. Therefore, we included all twenty-one subjects (regardless of contingency awareness ratings) and performed secondary sensitivity analyses for our main findings on Day 2 fear and extinction recall.

Consistent with the analyses reported above, differential BOLD responses were significantly reduced for extinguished versus nonextinguished stimuli [(CS+N – CS-N) > (CS+E – CS-E)] in the left amygdala (peak voxel in MNI space: $X = -22$ mm, $Y = -8$ mm, $Z = -16$ mm, $P_{FWE} = 0.032$). However, when all subjects were included in the analysis on frontomedial EEG theta power, the *Contingency* x *Extinction* interaction did not reach significance, $F(1,20) = 2.04$, $P = 0.169$. In addition, when theta FER scores were entered as covariate in second-level simple regression fMRI analysis, no association between EEG and fMRI data could be observed.

In summary, mirroring previous findings (Tabbert et al. 2011), our additional sensitivity analyses suggest that amygdala activation is less susceptible to an absence of subjective CS-US contingency awareness than our other measures of the conditioned response.

I Trial-By-Trial Coupling of EEG Theta Oscillations and fMRI Activation

Covariation of theta oscillations and amygdala activation, observed as a between subject correlation, is considered the main contribution of this study. However, investigating theta-amygdala coupling *within* subjects may provide even more relevant information with regard to functional interactions of different neural systems (Mueller, Stemmler, Wacker 2010). To gain more insight into these mechanisms, we computed an additional first-level GLM which allows to observe trial-by-trial coupling of EEG theta oscillations and fMRI activation (Debener et al. 2005).

Instead of four task-related regressors (i.e., CS+E, CS+N, CS-E, CS-N), this additional first-level GLM contained only one regressor indicating the onsets of all CS presentations, regardless of the CS type (Debener et al. 2005). Referring to these CS onsets, an additional regressor which represented the ln-transformed single-trial EEG theta power vector was added in the first-level

model as parametric modulator (Büchel et al. 1996; Büchel et al. 1998a). EEG epochs containing artifacts were replaced by the mean theta power value of the corresponding CS type for each subject. Thus, the single-trial EEG theta power vector was used to predict the fMRI BOLD signal. Specifically, the central goal of this additional analysis was to test whether hemodynamic signals covaried with the single-trial theta power within subjects (Debener et al. 2005). However, when this parametric regressor, which represents the single-trial EEG theta power vector, was tested against zero during the second-level group analysis, we could not detect any brain region that showed a significant covariation with EEG theta power.

Although we assume that amygdala BOLD responses (fMRI) and frontomedial oscillatory theta activity (EEG) are mechanistically connected, detecting a coupling of both measures on a trial-by-trial level is challenging, as they show a different habituation gradient over time. Given that amygdala activation habituates relatively quickly (e.g., Büchel et al. 1998b), the number of trials with a robust amygdala signal which are available for single trial analyses is dramatically reduced. However, analyses on EEG theta oscillations require a relatively high number of trials to achieve an adequate signal-to-noise ratio (Huffmeijer et al. 2014), due to a high level of noise in single EEG epochs (Huster et al. 2012). To assess trial-by-trial coupling of EEG theta oscillations and fMRI amygdala activation, a more habituation-resistant conditioning and extinction design would be required. One possibility to address this issue would be to present multiple different faces per CS type (e.g., five different CS+E etc.), rather than one face for each affective category (e.g., so-called MultiCS Conditioning, Steinberg et al. 2013). Contrary to traditional conditioning paradigms, this modified approach would (1) provide a good signal-to-noise ratio for EEG analyses, and further (2) avoid many repetitions of each CS face. Consequently, strong attenuation of amygdala activation after many CS presentations due to rapid habituation processes can be avoided, which would be required to assess theta-amygdala covariation at the trial-by-trial level.

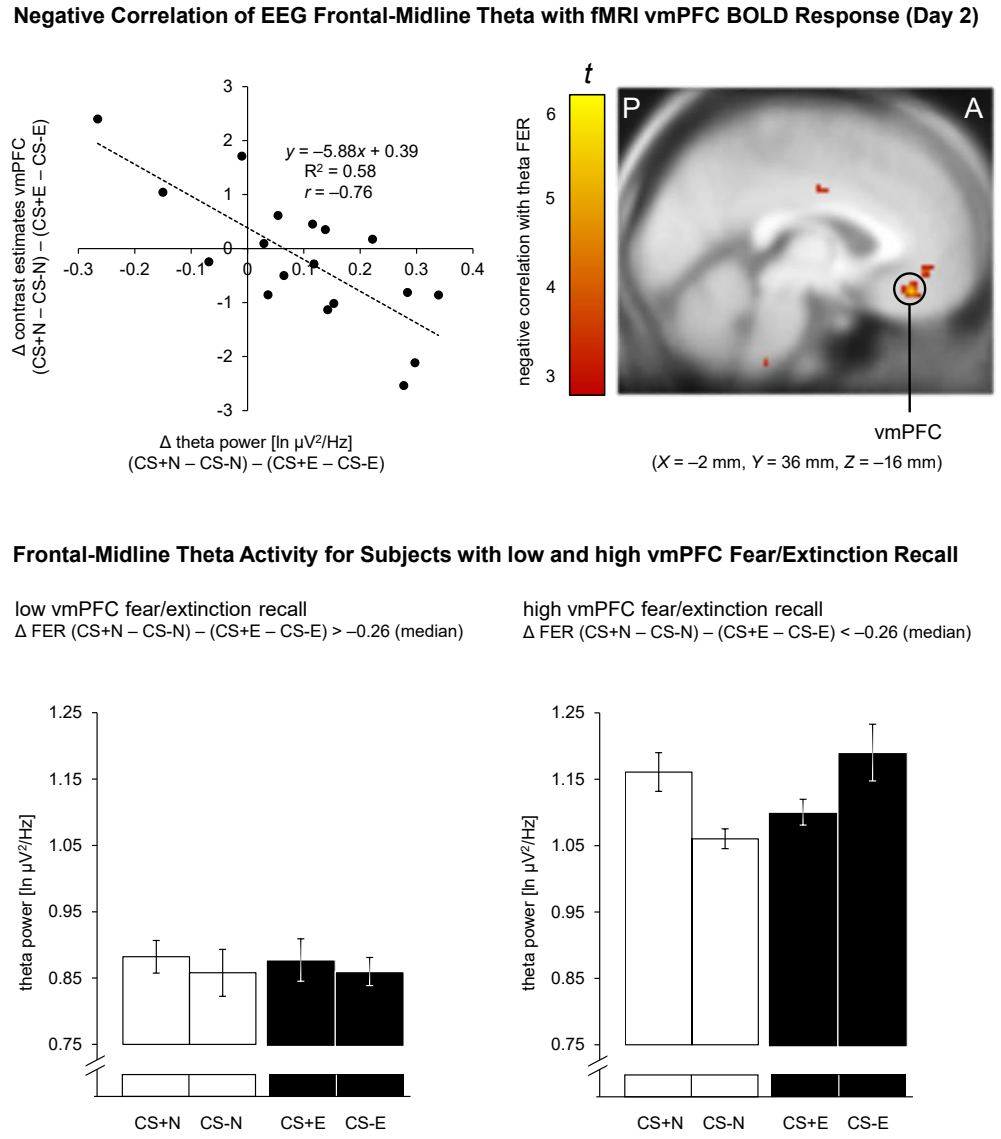
Supplementary Table S1. Day 2 Recall Test, restricted to first four trials: Localization and statistics of the peak voxels of significant activations for fear and extinction recall within previously defined ROIs (one-sample *t*-tests and correlations with EEG Theta FER[a])

Experimental phase	Brain Structure	Side	MNI Coordinates *X*	*Y*	*Z*	t_{max}	P_{FWE}
Day 2 Fear and Extinction Recall Test, restricted to first four trials (Milad et al. 2007)							
(CS+N – CS-N) > (CS+E – CS-E)	Amygdala	L	-26	-8	-18	4.23	0.036*
	Amygdala	R	30	-6	-20	4.17	0.043*
Positive Corr. with EEG Theta FER[a]	Amygdala	R	32	0	-22	3.89	0.087^{+}
Negative Corr. with EEG Theta FER[a]	vmPFC	L	-2	36	-16	5.63	0.038*
(CS+N – CS-N) < (CS+E – CS-E)[b]	– No significant results –						

[a]EEG Theta FER = frontomedial (electrode Fz) theta fear and extinction recall assessed by the tetrad contrast (CS+N – CS-N) – (CS+E – CS-E)

[b]Note that correlations of this BOLD contrast with EEG Theta FER scores are not displayed separately, as these correlations are already covered by the correlations listed above. For example, a positive correlation of Theta FER scores with the contrast (CS+N – CS-N) > (CS+E – CS-E) is equivalent to a negative correlation with the contrast (CS+N – CS-N) < (CS+E – CS-E).

$^{+}P_{FWE} \leq 0.10$, $*P_{FWE} \leq 0.05$, $**P_{FWE} \leq 0.01$ (ROI analyses, FWE-corrected according to SPM12 small volume correction, one peak per cluster is listed). All coordinates (*X*, *Y*, *Z*) are given in MNI space. L = left, R = right brain hemisphere.



Supplementary Figure S5. Integration of EEG frontomedial (Fz) theta power and fMRI vmPFC activation of fear and extinction recall on Day 2. (A) Negative correlation of theta modulations to conditioned and extinguished fear with BOLD responses in the vmPFC. Consistent with the assumed involvement of vmPFC in fear extinction recall, the correlation indicates that subjects with relatively strong vmPFC activation to extinguished (vs. nonextinguished) fear stimuli are characterized by relatively suppressed frontomedial theta power to extinguished (vs. nonextignuished) fear stimuli. For illustration purposes, the intensity threshold was set to $P \leq 0.005$ (uncorrected) with a minimal cluster threshold of $k \geq 5$ contiguous significant voxels. Activations (t-values) were superimposed on the MNI305 T1 template. All coordinates (X, Y, Z) are given in MNI space. A = anterior, P = posterior. (B) In order to unravel the negative correlation, vmPFC BOLD responses for the FER contrast (CS+N – CS-N) – (CS+E – CS-E) were divided using a median split procedure, and theta power was assessed separately for subjects with low vmPFC fear/extinction recall (i.e., high FER BOLD scores) and high vmPFC fear/extinction recall (i.e., low FER BOLD scores), bar graphs indicate $M \pm$ within-subject *SEM* (O'Brien and Cousineau, 2014). Only for subjects showing a large vmPFC extinction recall, differential frontomedial theta power for nonextinguished stimuli was significantly enhanced compared to extinguished stimuli ($P = 0.001$). Conversely, differential theta responses did not differ for subjects with low vmPFC extinction recall ($P = 0.893$).

Supplementary Table S2. Day 2 Recall Test, multivariate regression analysis on the covariation of fear-related (CS+N minus CS-N) and extinction-related (CS+E minus CS-E) signatures on EEG frontomedial theta oscillations and fMRI right amygdala activation

	fMRI Right Amygdala Activation	
EEG Theta Power	(CS+N – CS-N)	(CS+E – CS-E)
Multivariate regression analysis		
(CS+N – CS-N)	$F(2,14) = 8.31$, $P = 0.004$**	
(CS+E – CS-E)	$F(2,14) = 3.34$, $P = 0.065^{+}$	
Univariate follow-up multiple regression analyses		
(CS+N – CS-N)	$\beta = -0.20$, $P = 0.417$	$\beta = -0.74$, $P = 0.001$**
(CS+E – CS-E)	$\beta = -0.31$, $P = 0.216$	$\beta = 0.35$, $P = 0.064^{+}$

$^{+}P \leq 0.10$, $*P \leq 0.05$, $**P \leq 0.01$

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Yin, S., Liu, Y., Petro, N. M., Keil, A., & Ding, M. (2018). Amygdala adaptation and temporal dynamics of the salience network in conditioned fear: A single-trial fMRI study. *eNeuro*, *5*, 0445-17. https://doi.org/10.1523/ENEURO.0445-17.2018

Zeidan, M. A., Igoe, S. A., Linnman, C., Vitalo, A., Levine, J. B., Klibanski, A., Goldstein, J. M., & Milad, M. R. (2011). Estradiol modulates medial prefrontal cortex and amygdala activity during fear extinction in women and female rats. *Biological Psychiatry*, *70*, 920–927. https://doi.org/10.1016/j.biopsych.2011.05.016

7 Appendix

7.1 Relative Contributions to the Publications

Relative contributions of Matthias F. J. Sperl (MFJS) to the included manuscripts:

Manuscript 1: *Relative contribution of MFJS = 60%*

Sperl, M. F. J., Panitz, C., Hermann, C., & Mueller, E. M. (2016). A pragmatic comparison of noise burst and electric shock US for fear conditioning research with many trials. *Psychophysiology*, *53*, 1352–1365. https://doi.org/10.1111/psyp.12677

Manuscript 2: *Relative contribution of MFJS = 25%*

Mueller, E. M., **Sperl, M. F. J.**, & Panitz, C. (2019). Aversive imagery causes de novo fear conditioning. *Psychological Science*, *30*, 1001–1015. https://doi.org/10.1177/0956797619842261

Manuscript 3: *Relative contribution of MFJS = 70%*

Sperl, M. F. J., Wroblewski, A., Mueller, M., Straube, B., & Mueller, E. M. (2021). Learning dynamics of electrophysiological brain signals during human fear conditioning. *NeuroImage*, *226*, 117569. https://doi.org/10.1016/j.neuroimage.2020.117569

Manuscript 4: *Relative contribution of MFJS = 65%*

Sperl, M. F. J., Panitz, C., Skoluda, N., Nater, U. M., Pizzagalli, D. A., Hermann, C., & Mueller, E. M. (submitted). Alpha-2 adrenoreceptor antagonist yohimbine potentiates consolidation of conditioned fear. *Submitted to Neuropsychopharmacology*.

Manuscript 5: *Relative contribution of MFJS = 10%*

Panitz, C., **Sperl, M. F. J.**, Hennig, J., Klucken, T., Hermann, C., & Mueller, E. M. (2018). Fearfulness, neuroticism/anxiety, and COMT Val158Met in long-term fear conditioning and extinction. *Neurobiology of Learning and Memory*, *155*, 7–20. https://doi.org/10.1016/j.nlm.2018.06.001

Manuscript 6: *Relative contribution of MFJS = 70%*

Sperl, M. F. J., Panitz, C., Rosso, I. M., Dillon, D. G., Kumar, P., Hermann, A., Whitton, A. E., Hermann, C., Pizzagalli, D. A., & Mueller, E. M. (2019). Fear extinction recall modulates human frontomedial theta and amygdala activity. *Cerebral Cortex*, *29*, 701–715. https://doi.org/10.1093/cercor/bhx353

7.2 Curriculum Vitae

Matthias F. J. Sperl (M. Sc.)

University of Marburg
Department of Psychology
Personality Psychology and Assessment
Gutenbergstraße 18
35032 Marburg, Germany
matthias.sperl@staff.uni-marburg.de
ORCID: https://orcid.org/0000-0002-5011-0780

SCIENTIFIC EDUCATION

Since 2016 **University of Marburg (Marburg, Germany): Doctoral Candidate**
Member of the Research Training Group (RTG) 2271,
"Breaking Expectations: Maintenance vs. Change of Expectations in the Context of Expectation Violations"

Supervisor:
Prof. Dr. Erik M. Mueller
University of Marburg
(Department of Psychology, Personality Psychology and Assessment)
Co-Advisors:
Prof. Dr. Diego A. Pizzagalli
Harvard Medical School & McLean Hospital (Boston, MA, USA)
(Department of Psychiatry; Center for Depression, Anxiety and Stress Research)
Prof. Dr. Christiane Hermann
University of Giessen
(Department of Psychology, Clinical Psychology and Psychotherapy)

2013 – 2016 **University of Giessen (Giessen, Germany):**
Master of Science Psychology
and PreProPsych Pre-Doctorate Program

Overall grade: "very good with distinction" (0.9)

2010 – 2013 **University of Giessen (Giessen, Germany):**
Bachelor of Science Psychology

Overall grade: "very good with distinction" (0.9)

2010 **Ortenburg-Gymnasium Oberviechtach (Oberviechtach, Germany):**
Abitur (German university entrance qualification)

INTERNATIONAL RESEARCH EXPERIENCE: HARVARD MEDICAL SCHOOL (USA)

02/2018 – 08/2018 **Harvard Medical School, McLean Hospital (Boston, MA, USA)**
Supervisor: Prof. Dr. Diego A. Pizzagalli
Six-month research stay

08/2016 – 10/2016 **Harvard Medical School, McLean Hospital (Boston, MA, USA)**
Supervisor: Prof. Dr. Diego A. Pizzagalli
Two-month research stay

08/2015 – 01/2016 **Harvard Medical School, McLean Hospital (Boston, MA, USA)**
Supervisor: Prof. Dr. Diego A. Pizzagalli
Six-month research stay

As part of my research projects, I have collaborated with Prof. Dr. Diego A. Pizzagalli, Prof. Dr. Kerry J. Ressler, Prof. Dr. Isabelle M. Rosso, Prof. Dr. Daniel G. Dillon, Prof. Dr. Poornima Kumar, Dr. Antonia V. Seligowski, and Dr. Alexis E. Whitton.

TRAINING AS A PSYCHOLOGICAL PSYCHOTHERAPIST: UNIVERSITY OF GIESSEN

Since 2018 **University of Giessen (Giessen, Germany):**
Training as a Psychological Psychotherapist
Postgraduate training, focus on behavior therapy (Prof. Dr. Rudolf Stark)

10/2018 – 09/2019 **University Hospital of Giessen and Marburg, Center for Psychiatry and Psychotherapy Giessen (Giessen, Germany)**
Practical therapeutic work, as part of training as a psychological psychotherapist (Prof. Dr. Christoph Mulert)

SCIENTIFIC HONORS AND AWARDS

2019 **Research Training Group (RTG) 2271,**
"Breaking Expectations: Maintenance vs. Change of Expectations in the Context of Expectation Violations":
Student Poster Award
Poster Award for the contribution "Conditioned fear modulates event-related potential components during a sequential-set fear acquisition paradigm" (Awarded at the RTG Retreat in Hirschegg/Kleinwalsertal, Austria)

2018 **German Society for Basic and Applied Psychophysiology (Deutsche Gesellschaft für Psychophysiologie und ihre Anwendung; DGPA):**
Brain Products Young Scientist Award
Brain Products Young Scientist Award for a Distinguished Contribution in EEG Research, awarded for the publication "Fear extinction recall modulates human frontomedial theta and amygdala activity" (published in *Cerebral Cortex*) (Awarded at the DGPs/DGPA Annual Meeting "Psychology and the Brain" in Giessen, Germany)

2017 **Society for Psychophysiological Research (SPR):**
Student Poster Award

Poster Award for the contribution "Noradrenergic and dopaminergic modulation of long-term fear conditioning and extinction in humans"
(Awarded at the SPR Annual Meeting in Vienna, Austria)

2017 **World Association for Stress Related and Anxiety Disorders (WASAD):**
Young Researcher Award

WASAD Young Researcher Award for the project "Fear extinction recall modulates human frontomedial theta and amygdala activity during simultaneous EEG-fMRI"
(Awarded at the WASAD Annual Meeting in Wuerzburg, Germany)

GRANTS AND FELLOWSHIPS

2021 **Research Training Group (RTG) 2271,**
"Breaking Expectations: Maintenance vs. Change of Expectations in the Context of Expectation Violations":
Treasure Box Award

Funding for a research project on expectation violations in fear extinction in collaboration with Dr. Karoline Körfer (University of Marburg)

2019 **Society for Psychophysiological Research (SPR):**
SPR Research Travel Award

Travel Award to attend the 59th Annual Meeting of the Society for Psychophysiological Research (Washington/DC, USA)

2018 **Society for Psychophysiological Research (SPR):**
Research Training Fellowship

Research Training Fellowship for a six-month research stay at McLean Hospital, Harvard Medical School (Boston, MA, USA)

2016 **German Academic Exchange Service**
(Deutscher Akademischer Austauschdienst; DAAD):
Marburg International Doctorate

Travel Award to attend the 56th Annual Meeting of the Society for Psychophysiological Research (Minneapolis, MN, USA)

2016 **German Psychological Society**
(Deutsche Gesellschaft für Psychologie; DGPs):
Section for Personality Psychology and Psychological Diagnostics

Scholarship for a doctoral students workshop,
Contribution: "Neurobiological mechanisms of personality differences in fear extinction" (Landau/Pfalz, Germany)

2015 – 2016 **German Academic Exchange Service**
(Deutscher Akademischer Austauschdienst; DAAD):
PROMOS Scholarship

PROMOS Scholarship for a six-month research stay at McLean Hospital, Harvard Medical School (Boston, MA, USA)

2015 **Society for Psychophysiological Research (SPR): Student Travel Award**

Travel Award to attend the 55th Annual Meeting of the Society for Psychophysiological Research (Seattle, WA, USA)

PUBLICATIONS

Sperl, M. F. J., Panitz, C., Skoluda, N., Nater, U. M., Pizzagalli, D. A., Hermann, C., & Mueller, E. M. (submitted). Alpha-2 adrenoreceptor antagonist yohimbine potentiates consolidation of conditioned fear. Submitted to *Neuropsychopharmacology*.

Göhler, A. C., Haas, J. W., **Sperl, M. F. J.**, Hermann, C., & Winkler, A. (major revision). Placebo nasal spray protects female participants from experimentally induced sadness and concomitant changes in autonomous arousal. Major Revision at *Journal of Affective Disorders*.

Panitz, C., Endres, D., Buchholz, M., Khosrowtaj, Z., **Sperl, M. F. J.**, Mueller, E. M., Schubö, A., Schütz, A. C., Teige-Mocigemba, S., & Pinquart, M. (submitted). A revised framework for the investigation of expectation update versus maintenance in the context of expectation violations: The ViolEx 2.0 model. Submitted to *Frontiers in Psychology*. Preprint available at *PsyArXiv*. https://doi.org/10.31234/osf.io/vxs7y

Bierwirth, P., **Sperl, M. F. J.**, Antov, M. I., & Stockhorst, U. (in press). Prefrontal theta oscillations are modulated by estradiol-status during fear recall and extinction recall. *Biological Psychiatry: Cognitive Neuroscience and Neuroimaging*. https://doi.org/10.1016/j.bpsc.2021.02.011

Sperl, M. F. J., Wroblewski, A., Mueller, M., Straube, B., & Mueller, E. M. (2021). Learning dynamics of electrophysiological brain signals during human fear conditioning. *NeuroImage*, *226*, 117569. https://doi.org/10.1016/j.neuroimage.2020.117569
Open Data and Open Materials available online at Zenodo:
https://doi.org/10.5281/zenodo.4294603

Mueller, E. M., **Sperl, M. F. J.**, & Panitz, C. (2019). Aversive imagery causes de novo fear conditioning. *Psychological Science*, *30*, 1001–1015. https://doi.org/10.1177/0956797619842261
Open Data and Open Materials available online at Zenodo:
https://doi.org/10.5281/zenodo.2591593

Sperl, M. F. J., Panitz, C., Rosso, I. M., Dillon, D. G., Kumar, P., Hermann, A., Whitton, A. E., Hermann, C., Pizzagalli, D. A., & Mueller, E. M. (2019). Fear extinction recall modulates human frontomedial theta and amygdala activity. *Cerebral Cortex*, *29*, 701–715. https://doi.org/10.1093/cercor/bhx353

Panitz, C., **Sperl, M. F. J.**, Hennig, J., Klucken, T., Hermann, C., & Mueller, E. M. (2018). Fearfulness, neuroticism/anxiety, and COMT Val158Met in long-term fear conditioning and extinction. *Neurobiology of Learning and Memory*, *155*, 7–20. https://doi.org/10.1016/j.nlm.2018.06.001

Sperl, M. F. J., Panitz, C., Hermann, C., Mueller, E. M. (2016). A pragmatic comparison of noise burst and electric shock US for fear conditioning research with many trials. *Psychophysiology*, *53*, 1352–1365. https://doi.org/10.1111/psyp.12677

CONFERENCE CONTRIBUTIONS: TALKS AND SYMPOSIA

Talk at the **61st Annual Meeting of the Society for Psychophysiological Research (SPR)**, Virtual Conference due to the COVID-19 Pandemic
Symposium: "Turning threat into safety: A variety of emotional manipulations and their underlying neuronal mechanisms." (Chairs: Dr. Barbara Schmidt, **Matthias F. J. Sperl**)

Sperl, M. F. J., Panitz, C., Skoluda, N., Nater, U. M., Pizzagalli, D. A., Hermann, C., Mueller, E. M. (2021). Pharmacological modulation of threat and safety: Noradrenaline potentiates conditioned fear bradycardia and N170/LPP ERP amplitudes.

Talk at the **46th Joint Annual Meeting "Psychology and the Brain"** of the Division of Biological Psychology and Neuropsychology of the German Psychological Society (DGPs) and the German Society for Basic and Applied Psychophysiology (DGPA),
Virtual Conference due to the COVID-19 Pandemic
Symposium: "Neurophysiology of aversive conditioning and (emotional) memory." (Chairs: Prof. Dr. Ursula Stockhorst, Prof. Dr. Andreas Keil)

Sperl, M. F. J., Panitz, C., Skoluda, N., Nater, U. M., Pizzagalli, D. A., Hermann, C., Mueller, E. M. (2021). Noradrenergic modulations of fear conditioning: Yohimbine potentiates fear-conditioned bradycardia, N170, and late positive potential amplitudes.

Talk at the **38th Meeting of the Division of Clinical Psychology and Psychotherapy** of the German Psychological Society (DGPs), Virtual Conference due to the COVID-19 Pandemic
Symposium: "Neurobiologische Grundlagen aversiver und traumatischer Erlebnisse: Von basalen Mechanismen zur klinischen Relevanz. [Neurobiological signatures of aversive and traumatic experiences: From basal mechanisms to clinical relevance.]" (Chair: PD Dr. Andrea Hermann)

Sperl, M. F. J., Wroblewski, A., Mueller, M., Straube, B., & Mueller, E. M. (2021). Wie entsteht Furcht im Gehirn? Lerndynamiken neuronaler Prozesse bei der Bedrohungsverarbeitung. [How does fear develop in the brain? Learning dynamics of neuronal processes in threat processing.]

Talk at the **45th Joint Annual Meeting "Psychology and the Brain"** of the Division of Biological Psychology and Neuropsychology of the German Psychological Society (DGPs) and the German Society for Basic and Applied Psychophysiology (DGPA) in Dresden (Germany)
Symposium: "An update on EEG recording and analyses methods in affective and social neuroscience." (Chair: Dr. Bastian Schiller)

Sperl, M. F. J., Wroblewski, A., Mueller, M., Straube, B., & Mueller, E. M. (2019). Applying data-driven randomization statistics to unravel the temporal unfolding of neurophysiological mechanisms during fear conditioning.

Talk at the **37th Meeting of the Division of Clinical Psychology and Psychotherapy** of the German Psychological Society (DGPs) in Erlangen (Germany)
Symposium: Young Scientist Symposium (Chairs: Dr. Lena Krämer, Dr. Jakob Fink)

Sperl, M. F. J., Panitz, C., Skoluda, N., Nater, U. M., Pizzagalli, D. A., Hermann, C., Mueller, E. M. (2019). Pharmakologische Augmentation bei der Expositionsbehandlung: Lässt sich die langfristige Furchtkonditionierung und -extinktion durch den α2-Adrenozeptor-Antagonisten Yohimbin oder den D2-Rezeptorblocker Sulpirid modulieren? [Pharmacological augmentation of exposure therapy: Can long-term fear conditioning and extinction be modulated by the α2-adrenoceptor antagonist yohimbine or the D2-receptor blocker sulpiride?]

Talk at the **45th Joint Annual Meeting "Psychology and the Brain"** of the Division of Biological Psychology and Neuropsychology of the German Psychological Society (DGPs) and the German Society for Basic and Applied Psychophysiology (DGPA) in Giessen (Germany); talk on the occasion of the awarding of the **Brain Products Young Scientist Award for a Distinguished Contribution in EEG Research** (Chair: Prof. Dr. Martin J. Herrmann)

Sperl, M. F. J., Panitz, C., Rosso, I. M., Dillon, D. G., Kumar, P., Hermann, A., Whitton, A. E., Hermann, C., Pizzagalli, D. A., & Mueller, E. M. (2018). Fear extinction recall modulates human frontomedial theta and amygdala activity.

Talk at the **Congress of the World Association for Stress Related and Anxiety Disorders** in Wuerzburg (Germany); talk on the occasion of the awarding of the **WASAD Young Researcher Award** (Chairs: Prof. Dr. Peter Riederer, Prof. Dr. Christian Büchel, Dr. Tina B. Lonsdorf)

Sperl, M. F. J., Panitz, C., Rosso, I. M., Dillon, D. G., Kumar, P., Hermann, A., Whitton, A. E., Hermann, C., Pizzagalli, D. A., & Mueller, E. M. (2017). Fear extinction recall modulates human frontomedial theta and amygdala activity during simultaneous EEG-fMRI. *Journal of Neural Transmission, 124*, 1304.

Talks at the **fMRI Methods Meeting** "New Directions in Psychological Research Using Functional Magnetic Resonance Imaging" in Rauischholzhausen (Germany), organized by the Bender Institute of Neuroimaging Giessen

Sperl, M. F. J. (2017). *DO*s and *DON'T*s of data analysis … in times of *p*-hacking and replication crisis.

Sperl, M. F. J. (2017). (How) shall we scan on?! Validity of statistical tests in fMRI research.

CONFERENCE CONTRIBUTIONS: POSTER PRESENTATIONS

Sperl, M. F. J., Panitz, C., Skoluda, N., Nater, U. M., Pizzagalli, D. A., Hermann, C., Mueller, E. M. (2020). Noradrenaline potentiates conditioned fear bradycardia, N170, and late positive potential amplitudes. *UNSW Workshop on Expectation, Perception & Cognition.* (University of New South Wales; Virtual Conference due to the COVID-19 Pandemic)

Sperl, M. F. J., Panitz, C., Skoluda, N., Nater, U. M., Pizzagalli, D. A., Hermann, C., Mueller, E. M. (2019). Pharmacological modulation of conditioned fear: Alpha-2 adrenoreceptor antagonist yohimbine potentiates fear bradycardia and N170 ERP amplitude. *EPFL Neuro Symposium "Fear Learning – From Neuronal Circuits to Translation".* (École polytechnique fédérale de Lausanne; Lausanne, Switzerland)

Sperl, M. F. J., Panitz, C., Skoluda, N., Nater, U. M., Pizzagalli, D. A., Hermann, C., Mueller, E. M. (2019). Alpha-2 adrenoreceptor antagonist yohimbine modulates consolidation of conditioned fear. *Science Day of the Research Campus Central Hessen; Center for Mind, Brain and Behavior.* (Rauischholzhausen, Germany)

Sperl, M. F. J., Wroblewski, A., Mueller, M., Straube, B., & Mueller, E. M. (2019). Temporal dynamics of threat processing: Modulation of event-related potential components during a sequential-set fear acquisition paradigm. *Journal of Neural Transmission, 126*, 1554. *International Congress of the World Association for Stress Related and Anxiety Disorders, WASAD.* (Wuerzburg, Germany)

Sperl, M. F. J., Wroblewski, A., Mueller, M., Straube, B., & Mueller, E. M. (2019). Validation of a new sequential-set fear acquisition paradigm: Conditioned fear modulates early and late event-related potential components. *Psychophysiology, 56*, S112. (Society for Psychophysiological Research; Washington/DC, USA)

Sperl, M. F. J., Wroblewski, A., Mueller, M., Straube, B., & Mueller, E. M. (2019). Conditioned fear modulates event-related potential components during a sequential-set fear acquisition paradigm. *Retreat of the Research Training Group (RTG) 2271.* (Hirschegg/Kleinwalsertal, Austria)

Sperl, M. F. J., Wroblewski, A., Mueller, M., Straube, B., & Mueller, E. M. (2019). Conditioned fear modulates event-related potential components during a sequential-set fear acquisition paradigm. *European Meeting on Human Fear Conditioning.* (Wuerzburg, Germany)

Sperl, M. F. J., Panitz, C., Skoluda, N., Nater, U. M., Hermann, C., Mueller, E. M. (2017). α2-adrenoreceptor antagonist yohimbine modulates consolidation of conditioned fear. *Psychophysiology, 54*, S90. (Society for Psychophysiological Research; Wien, Austria)

Sperl, M. F. J., Panitz, C., Skoluda, N., Nater, U. M., Hermann, C., Mueller, E. M. (2017). α2-adrenoreceptor antagonist yohimbine modulates consolidation of conditioned fear. *European Meeting on Human Fear Conditioning.* (Hamburg, Germany)

Sperl, M. F. J., Panitz, C., Rosso, I. M., Dillon, D. G., Whitton, A. E., Kumar, P., Hermann, A., Hermann, C., Pizzagalli, D. A., & Mueller, E. M. (2016). Validation of an experimental paradigm for simultaneous fMRI-EEG: Modulation of theta oscillations by fear conditioning and extinction. *Psychophysiology, 53*, S94. (Society for Psychophysiological Research; Minneapolis, MN, USA)

Sperl, M. F. J., Panitz, C., Rosso, I. M., Dillon, D. G., Whitton, A. E., Kumar, P., Hermann, A., Hermann, C., Pizzagalli, D. A., & Mueller, E. M. (2016). Validierung eines experimentellen Paradigmas zur simultanen fMRT-EEG-Messung: Modulation von Theta-Oszillationen durch konditionierte und extingierte Furcht. [Validation of an experimental paradigm for simultaneous fMRI-EEG: Modulation of theta oscillations by conditioned and extinguished fear.] *Psychology and the Brain*. (German Psychological Society, DGPs; and German Society for Basic and Applied Psychophysiology, DGPA; Berlin, Germany)

Sperl, M. F. J., Panitz, C., Whitton, A. E., Rosso, I. M., Dillon, D. G., Kumar, P., Hermann, A., Hermann, C., Pizzagalli, D. A., & Mueller, E. M. (2016). Integration of hemodynamic and electrophysiological correlates of human long-term fear conditioning and extinction by simultaneous EEG-fMRI. *European Meeting on Human Fear Conditioning*. (Utrecht, Netherlands)

Sperl, M. F. J., Panitz, C., Rosso, I. M., Dillon, D. G., Kumar, P., Hermann, A., Hermann, C., Pizzagalli, D. A., & Mueller, E. M. (2016). Modulation of theta oscillations by fear conditioning and extinction during simultaneous EEG and fMRI. *McLean Research Day, McLean Hospital / Harvard Medical School*. (Belmont, MA, USA)

Sperl, M. F. J., Panitz, C., Hermann, C., Mueller, E. M. (2015). Electric shock versus white noise burst: Which US-type is better for fear conditioning with many trials? *Psychophysiology, 52*, S93. (Society for Psychophysiological Research; Seattle, WA, USA)

Sperl, M. F. J., Panitz, C., Hermann, C., Mueller, E. M. (2015). Elektrischer Reiz vs. weißes Rauschen: Welcher US eignet sich besser zur Furchtkonditionierung mit vielen Lerndurchgängen? [Electric shock vs. white noise burst: Which US-type is better for fear conditioning with many trials?] *Psychology and the Brain*. (German Psychological Society, DGPs; and German Society for Basic and Applied Psychophysiology, DGPA; Frankfurt/Main, Germany)

PEER REVIEW ACTIVITIES

Biological Psychiatry: Cognitive Neuroscience and Neuroimaging
Cognition and Emotion
European Journal of Neuroscience
International Journal of Psychophysiology
NeuroImage
Psychiatry Research: Neuroimaging
Psychophysiology
Scientific Reports

TEACHING

Communication Skills and Diagnostics in Clinical Psychology (4 courses)

Affective Disorders (4 courses)

Communication Skills, Psychodiagnostic Interview, and Behavioral Monitoring (3 courses)

Anxiety Disorders (2 courses)

Personality and Negative Affect (1 course)

Supervision of 17 Bachelor of Science theses and 7 Master of Science theses

INVOLVEMENT IN PROFESSIONAL SOCIETIES

Society for Psychophysiological Research (SPR)
Society Involvement:
Chair, International Students Subcommittee (since 2017)
Chair, Student Meeting Events Subcommittee, Vienna Meeting (2016–2017)
Committee Member, International Students Subcommittee (since 2016)
Committee Member, Committee to Promote Student Interests (since 2016)
Committee Member, Program Committee, Meeting 2021 Virtual (2020–2021)
Committee Member, Program Committee, Meeting 2020 Virtual (2019–2020)
Committee Member, Program Committee, Meeting 2019 Washington/DC (2018–2019)

German Psychological Society (Deutsche Gesellschaft für Psychologie; DGPs)
Society Involvement:
Deputy Representative of Young Members, Division of Biological Psychology and Neuropsychology (since 2021)
Committee Member, Young Scientists Subcommittee “Reform of Psychotherapy Training in Germany,” Division of Clinical Psychology and Psychotherapy (since 2018)
Member of four divisions:
Division of Biological Psychology and Neuropsychology
Division of Clinical Psychology and Psychotherapy
Division of Personality Psychology and Psychological Diagnostics
Division of General Psychology

German Society for Basic and Applied Psychophysiology (Deutsche Gesellschaft für Psychophysiologie und ihre Anwendung; DGPA)
Society Involvement:
Internal Auditor ("Kassenprüfer," 2019–2021)

Association for Psychological Science (APS)

Society for Neuroscience (SfN)

World Association for Stress Related and Anxiety Disorders (WASAD)

European Association for Clinical Psychology and Psychological Treatment (EACLIPT)

International Exchange Alumni Community
(United States Department of State, Bureau of Educational and Cultural Affairs)

Center for Mind, Brain and Behavior (CMBB), Research Campus Central Hessen

Marburg University Research Academy

Giessen University Society (Gießener Hochschulgesellschaft)

Research Internships and Experience as Student Research Assistant

10/2012 – 02/2016	**Student Research Assistant, University of Giessen (Germany)** **Department of Clinical Psychology and Psychotherapy** (Prof. Dr. Christiane Hermann)
07/2013 – 07/2015	**Student Research Assistant, University of Giessen (Germany)** **Local Ethics Committee** **of the Faculty of Psychology and Sports Science** (Prof. Dr. Christiane Hermann, Prof. Dr. Karsten Krüger)
03/2013 – 08/2013	**Student Research Assistant, University of Giessen (Germany)** **Department of Educational Psychology** (Prof. Dr. Kristin Krajewski)
10/2012 – 04/2013	**Research Internship, University of Giessen (Germany)** **Bender Institute of Neuroimaging** (Prof. Dr. Rudolf Stark)

Clinical Internships

08/2014 – 09/2014	**Psychiatric University Hospital Zurich (Zurich, Switzerland)** (Prof. Dr. Heinz Böker)
07/2013 – 09/2013	**Barmherzige Brüder Graz-Eggenberg, Residential Drug Addiction Treatment Center "Walkabout" (Kainbach/Graz, Austria)** (Prim. Dr. Werner Friedl)

09/2012 – 10/2012 **University Hospital of Giessen and Marburg, Center for Psychiatry and Psychotherapy Giessen (Giessen, Germany)**
(Prof. Dr. Bernd Gallhofer)

02/2012 – 04/2012 **Hospital of Psychiatry, Psychotherapy and Psychosomatics "Hohe Mark" (Oberursel, Frankfurt/Main, Germany)**
(Prof. Dr. Arnd Barocka)

07/2010 – 09/2010 **Barmherzige Brüder Reichenbach, Residential and Day Services for Persons with Disabilities (Reichenbach, Germany)**

From 2012 to 2015, I volunteered as an instructor for the weekly **German Skills Training** at the **Center for Psychiatry and Psychotherapy** at the **University Hospital of Giessen and Marburg** (Giessen site, Prof. Dr. Bernd Gallhofer and Dr. Nina Haible-Baer). The aim of that group training was to teach language and cultural skills to patients with a migration background or native speakers of a language other than German.

7.3 Statement / Erklärung

I hereby declare that I have prepared my dissertation

Electrophysiological Signatures of Fear Conditioning: From Methodological Considerations to Catecholaminergic Mechanisms and Translational Perspectives

Elektrophysiologische Korrelate von Furchtkonditionierung: Von methodischen Überlegungen zu katecholaminergen Mechanismen und translationalen Perspektiven

independently and without unauthorized assistance, and that I have not used any sources or assistance other than those explicitly indicated by me.

The dissertation has not been submitted to any other university in its present or similar form and has not served any other examination purposes.

Hiermit erkläre ich, dass ich meine Dissertation

Electrophysiological Signatures of Fear Conditioning: From Methodological Considerations to Catecholaminergic Mechanisms and Translational Perspectives

Elektrophysiologische Korrelate von Furchtkonditionierung: Von methodischen Überlegungen zu katecholaminergen Mechanismen und translationalen Perspektiven

selbstständig und ohne unerlaubte Hilfe angefertigt habe und mich dabei keiner anderen als der von mir ausdrücklich bezeichneten Quellen und Hilfen bedient habe.

Die Dissertation wurde in der jetzigen oder einer ähnlichen Form noch bei keiner anderen Hochschule eingereicht und hat noch keinen sonstigen Prüfungszwecken gedient.

Marburg, den 09.07.2021

Matthias F. J. Sperl

University of Marburg
Department of Psychology
Personality Psychology and Assessment
Gutenbergstraße 18
35032 Marburg, Germany
matthias.sperl@staff.uni-marburg.de