Developmental Aspects of Fear Learning and Fear Generalization

Entwicklungsaspekte des Furchterwerbs und der Furchtgenerealisierung

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Abstract

In situations of real threat, showing a fear reaction makes sense, thus, increasing the chance to survive. The question is, how could anybody differentiate between a real and an apparent threat? Here, the slogan counts "better safe than sorry", meaning that it is better to shy away once too often from nothing than once too little from a real threat. Furthermore, in a complex environment it is adaptive to *generalize* from one threatening situation or stimulus to another similar situation/stimulus. But, the danger hereby is to generalize in a maladaptive manner involving as it is to strong and/or fear too often "harmless" (safety) situations/stimuli, as it is known to be a criterion of anxiety disorders (AD). *Fear conditioning* and *fear generalization* paradigms are well suited to investigate fear learning processes. It is remarkable that despite increasing interest in this topic there is only little research on fear generalization. Especially, most research on human fear conditioning and its generalization has focused on adults, whereas only little is known about these processes in children, even though AD is typically developing during childhood. To address this knowledge gap, four experiments were conducted, in which a discriminative fear conditioning and generalization paradigm was used.

In the first two experiments, developmental aspects of fear learning and generalization were of special interest. Therefore, in the first experiment 267 children and 285 adults were compared in the *differential fear conditioning* paradigm and generalization test. Skin conductance responses (SCRs) and ratings of valence and arousal were obtained to indicate fear learning. Both groups displayed robust and similar differential conditioning on subjective and physiological levels. However, children showed heightened fear generalization compared to adults as indexed by higher arousal ratings and SCRs to the generalization stimuli. Results indicate *overgeneralization* of conditioned fear as a developmental correlate of fear learning. The developmental change from a shallow to a steeper generalization gradient is likely related to the maturation of brain structures that modulate efficient discrimination between threatening and (ambiguous) safety cues. The question hereby is, at which developmental stage fear generalization gradients of children adapt to the gradients of adults. Following up on this question, in a second experiment, developmental changes in fear conditioning and fear generalization between children and adolescents were investigated. According to experiment 1 and previous studies in children, which showed changes in fear learning with increasing age, it was assumed that older children were better at discriminating threat and safety stimuli.

Therefore, 396 healthy participants (aged 8 to 12 years) were examined with the fear conditioning and generalization paradigm. Again, ratings of valence, arousal, and SCRs were obtained. SCRs indicated differences in fear generalization with best fear discrimination in 12-year-old children suggesting that the age of 12 years seems to play an important role, since generalization gradients were similar to that of adults. These age differences were seen in boys and girls, but best discrimination was found in 12-year-old boys, indicating different development of generalization gradients according to sex. This result fits nicely with the fact that the prevalence of AD is higher in women than in men.

In a third study, it was supposed that the developmental trajectory from increased trait anxiety in childhood to manifest AD could be mediated by abnormal fear conditioning and generalization processes. To this end, 394 children aged 8 to 12 years with different scores in trait anxiety were compared with each other. Results provided evidence that children with high trait anxiety showed stronger responses to threat cues and impaired safety signal learning contingent on awareness as indicated by arousal at acquisition. Furthermore, analyses revealed that children with high trait anxiety showed overall higher arousal ratings at generalization. Contrary to what was expected, high trait anxious children did not show significantly more fear generalization than children with low trait anxiety. However, high-trait-anxious (HA) participants showed a trend for a more linear gradient, whereas moderate-trait-anxious (MA) and low-trait-anxious (LA) participants showed more quadratic gradients according to arousal. Additionally, after controlling for age, sex and negative life experience, SCR to the safety stimulus predicted the trait anxiety level of children suggesting that impaired safety signal learning may be a risk factor for the development of AD.

Results provide hints that frontal maturation could develop differently according to trait anxiety resulting in different stimuli discrimination. Thus, in a fourth experiment, 40 typically developing volunteers aged 10 to 18 years were screened for trait anxiety and investigated with the differential fear conditioning and generalization paradigm in the scanner. Functional magnetic resonance imaging (fMRI) were used to identify the neural mechanisms of fear learning and fear generalization investigating differences in this neural mechanism according to trait anxiety, developmental aspects and sex. At acquisition, HA participants showed reduced activation in frontal brain regions, but at generalization, HA participants showed an increase in these frontal regions with stronger linear increase in activation with similarity to CS+ in HA when compared to LA participants. This indicates that there is a hyper-regulation in adolescents

to compensate the higher difficulties at generalization in form of a compensatory mechanism, which decompensates with adulthood and/or may be collapsed in manifest AD. Additionally, significant developmental effects were found: the older the subjects the stronger the hippocampus and frontal activation with resemblance to CS+, which could explain the overgeneralization of younger children. Furthermore, there were differences according to sex: males showed stronger activation with resemblance to CS+ in the hippocampus and frontal regions when compared to females fitting again nicely with the observation that prevalence rates for AD are higher for females than males.

In sum, the studies suggest that investigating developmental aspects of (maladaptive) overgeneralization may lead to better understanding of the mechanisms of manifest anxiety disorders, which could result in development and provision of prevention strategies. Although, there is need for further investigations, the present work gives some first hints for such approaches.

Zusammenfassung

Im Angesicht realer Bedrohung macht es durchaus Sinn, eine Furchtreaktion zu zeigen, denn dadurch kann die Überlebenschance erhöht werden. Die aufkommende Frage hierbei lautet: wie kann man eine "echte" Gefahr von einer "Attrappe" unterscheiden? Hier gilt der Spruch: besser einmal zu viel (Angst), als einmal zu wenig. Es macht also in einer komplexen Umwelt durchaus Sinn, von einer bestimmten Situation oder Gefahrenquelle auf eine andere, dieser ähnlichen, zu *generalisieren*. Dies birgt allerdings die Gefahr, zu stark zu generalisieren und/oder zu oft vor "harmlosen" Situationen oder Dingen Angst zu haben, wie uns das von den Angststörungen her bekannt ist. Furchterwerb und -generalisierung lassen sich mithilfe von Furchtkonditionierungs- und Generalisierungsparadigmen untersuchen. In Anbetracht der Tatsache, dass Furchtlernen und Furchtgeneralisierung wichtige Rollen in der Entwicklung von Angsterkrankungen zu spielen scheinen, ist es verwunderlich, dass es, trotz zunehmenden Interesses, nur wenig Forschung im Bereich Furchtgeneralisierung gibt. Die meisten Humanstudien hierzu wurden mit erwachsenen Probanden durchgeführt, wohingegen nur sehr wenig über diese Prozesse im Kindesalter bekannt ist. Um diese Wissenslücke etwas zu schließen, wurden in dieser vorliegenden Arbeit vier Studien generiert, in welchen ein differenzielles Furchtkonditionierungs- und Generalisierungsparadigma zum Einsatz kam.

In den ersten beiden Experimenten standen Entwicklungsaspekte des Furchterwerbs und der Furchtgeneralisierung im Fokus. In der ersten Studie haben wir deshalb 267 Kinder und 285 Erwachsene mit dem differenziellen Furchtkonditionierungs- und Generalisierungsparadigma untersucht und bezüglich Valenz, Arousal und Hautleitfähigkeitsreaktion verglichen. Während beide Gruppen vergleichbare Konditionierungseffekte aufwiesen, zeigten die Kinder eine stärkere Generalisierung. Dies machte sich sowohl im Arousal als auch in der Hautleitfähigkeit bemerkbar. Dieses Ergebnis lässt darauf schließen, dass eine Entwicklung von einem flachen hin zu einem steileren Generalisierungsgradienten möglicherweise auf die Reifung bestimmter Hirnstrukturen zurückzuführen ist, die die effektive Diskriminierung zwischen gefährlichen und (vermeintlich) ungefährlichen Reizen modulieren. Die Frage schließt sich an, in welchem Entwicklungsstadium sich die Generalisierungsgradienten von Kindern denen der Erwachsenen angleichen. Um dieser Frage weiter nachzugehen wurden in der zweiten Studie entwicklungsbedingte Veränderungen der Furchtkonditionierung und -generalisierung bei Kindern und Jugendlichen untersucht. Laut dem ersten Experiment und weiteren Studien, die

auf altersbedingte Veränderungen im Furchtlernen hinweisen, lag hier die Annahme zugrunde, dass Kinder mit zunehmendem Alter besser zwischen gefährlichen und (vermeintlich) ungefährlichen Reizen unterscheiden können. Um diese Hypothese zu überprüfen, wurden 396 gesunde Probanden (zwischen 8 und 12 Jahren) mittels des Furchtkonditionierungs- und Generalisierungsparadigmas untersucht. Wie bereits in der ersten Studie wurden Valenz, Arousal und die Hautleitfähigkeitsreaktion ausgewertet. Die Ergebnisse zeigten, dass Kinder aller Altersstufen vergleichbare Konditionierungseffekte aufweisen, dass aber laut der Hautleitfähigkeitsreaktion 12-jährige Kinder im Vergleich zu den jüngeren Kindern am besten zwischen den Stimuli unterscheiden konnten. Wenn nach Geschlecht aufgeteilt wird, diskriminierten 12-jährige Jungs am besten, was darauf schließen lässt, dass sich Furchtgeneralisierung je nach Geschlecht unterschiedlich entwickeln könnte, was in Anbetracht der Tatsache interessant ist, dass Frauen höhere Prävalenzraten für Angsterkrankungen haben als Männer.

Einer dritten Studie liegt die Annahme zugrunde, dass ein Entwicklungsstrang von einer ängstlichen Persönlichkeit hin zu einer Angsterkrankung durch abweichende Furchtkonditionierung und -Generalisierung vermittelt werden könnte. Daher wurden 394 Kinder zwischen 8 und 12 Jahren mit unterschiedlichen Ausprägungen in ihrer Ängstlichkeit mit dem Furchtkonditionierungs- und Generalisierungsparadigma untersucht. Die Ergebnisse zeigten, dass Kinder mit hohen Angst Werten je nach dem Grad ihrer Kontingenz-Bewusstheit während der Konditionierung mit stärkerer Erregung auf mit Gefahr assoziierte Reize und beeinträchtigtem Lernen des sicheren Reizes reagierten. Es zeigte sich, dass Kinder mit hohen Angst Werten generell höhere Erregungs-Werte während der Generalisierung aufwiesen als Kinder mit niedrigen Werten. Entgegen den Annahmen generalisierten Kinder mit hoher Ängstlichkeit nicht signifikant stärker als Kinder mit niedrigen Werten. Es gab jedoch einen Trend dahingehend, dass hoch-ängstliche Kinder eher lineare Generalisierungsgradienten aufweisen (was für mehr Generalisierung spricht), während moderat- und niedrig-ängstliche Kinder eher quadratische Gradienten haben (was für bessere Differenzierung spricht). Zudem konnten die SCRs auf den „sicheren“ Stimulus (CS-) den Ängstlichkeitswert voraussagen (nachdem für Alter, Geschlecht und negative Lebenserfahrung korrigiert wurde). Dies könnte bedeuten, dass eine Beeinträchtigung im Lernen des sicheren Reizes ein Risikofaktor für die Entwicklung einer Angsterkrankung im Jugendalter sein könnte.

Allgemein liefern die Ergebnisse erste Hinweise darauf, dass sich frontale Hirnstrukturen je nach Ausprägung der Ängstlichkeit unterschiedlich entwickeln könnten, was sich in unterschiedlicher Diskriminierungsfähigkeit spiegeln könnte. Um dem weiter nachzugehen, wurden im vierten Experiment 40 der Norm entsprechend entwickelte Probanden zwischen 10 und 18 Jahren mit unterschiedlichen hoher Ängstlichkeit mittels fMRT auf Unterschiede in der Furchtkonditionierung und -generalisierung hin untersucht. Außerdem wurden Zusammenhänge mit dem Alter und dem Geschlecht der Probanden untersucht. Die frontale Aktivität zeigte sich unterschiedlich je nach Ängstlichkeit, mit stärkerer linearer Zunahme in der Aktivierung mit Ähnlichkeit zum CS+ während der Generalisierung bei hoch ängstlichen Probanden. Dies lässt auf eine Hyperregulierung bei Jugendlichen im Vergleich zu Erwachsenen schließen, wenn es darum geht, die Schwierigkeiten bezüglich der Differenzierung der dem bedrohlichen Reiz sehr ähnlichen Reize zu kompensieren. Diese Hyperregulierung könnte einen Kompensationsmechanismus der hoch ängstlichen Kinder widerspiegeln, welcher mit zunehmendem Alter verschwindet und bei manifesten Angsterkrankungen zusammenbricht. Außerdem konnten signifikante Entwicklungseffekte gefunden werden: je älter die Probanden waren, desto stärker war deren Aktivierung im Hippocampus und in frontalen Regionen während der Generalisierung mit zunehmender Ähnlichkeit zum CS+. Diese Altersunterschiede könnten die Übergeneralisierung jüngerer Kinder erklären und folglich zur Erklärung beitragen, weshalb Angsterkrankungen häufig ihren Ursprung in frühen Altersstufen haben. Männliche Probanden zeigten außerdem stärkere Aktivierung mit zunehmender Ähnlichkeit zum CS+ im Hippocampus und in frontalen Regionen. Dieses Ergebnis ergänzt die Resultate des zweiten Experiments und passt zur Tatsache, dass die Prävalenzraten für Angsterkrankungen für Frauen höher sind als für Männer.

Zusammengenommen legen die Studien nahe, dass es sich lohnt, sich mit Entwicklungsaspekten von Furchtgeneralisierung zu befassen. Ein besseres Verständnis von Mechanismen des Furchterwerbs und besonders von Furchtgeneralisierung könnte dabei helfen, die Entstehungsmechanismen von Angsterkrankungen besser zu verstehen und könnte somit bei der Entwicklung von Präventionsansätzen wichtig sein. Obwohl viele Fragen noch offen sind und einige weitere Untersuchungen anstehen, liefert diese Arbeit erste Ideen für solche Ansätze.

1. Introduction

A citation of Johann Christoph Friedrich von Schiller (1798) "*Die Angst beflügelt den eilenden Fuß*" (*Die Bürgschaft*, strophe 15, rhyme 99 [free translated "fear inspires the hurrying feet"]) suggests that fear can elicit a meaningful reaction in the presence of a real threat. Fear is an aversive emotional state triggered by threatening cues activating the defensive fear system of an organism (Steimer, 2002). When realizing, for example, a creeping animal on the ground, it makes sense to shy away as quick as possible even before thinking about whether it could be a dangerous snake, or any other animal, or solely a stick. From an evolutionary point of view, it is better to shy away once too often from nothing than once too little from real threat, or in short: better safe than sorry. Thus, a fast, reflexive reaction, which works independently of aware thinking and planning, made an important contribution to survival in humans' ontogenesis. Therefore, such reflexive fear reactions have evolutionary asserted themselves until today, allowing an animal to be biologically prepared for danger-relevant cues (Öhman, 2005). These automatic processes do not require cognitive processing indicating a direct thalamus-amygdala connection (see chapter 1.2.3 for more details).

Considering this aspect from a developmental perspective, an adaption on an always changing environment with always new risk sources is essential to predict events and finally survive in such an environment. Thus, in an ever-changing environment learning plays an important role. If a child, for example, were bitten by a dog or observes such an event in another human, this child could show a certain reservation against dogs from now on and initiate a fear reaction in the presence of these animals. A differentiation of threat and safety cues in this context would be of crucial importance requiring a more cognitive conscious activity: Not only threat cues, but also safety cues have to be identified, because in the presence of safety cues you can relax for avoiding permanent arousal. Indeed, developmental studies of fear learning showed better discrimination between threat and safety cues with increasing age (e.g. Gao, Raine, Venables, Dawson, & Mednick, 2010; Glenn et al., 2012a) indicating that there is a maturational shift in fear learning.

In a complex environment, it is adaptive and could be an advantage to *generalize* from one stimulus to another similar stimulus. That means that a fear response could be elicited not only by the dangerous stimuli itself, but also by stimuli, which are similar to the dangerous stimuli. For example, from an evolutionary point of view it is meaningful to generalize from

one snake to another similar snake or from one big cat (e.g. a lion) to another big cat (e.g. a leopard). Studies of generalization throughout normal development revealed phases of higher susceptibility towards *fear generalization* qualified by age with less generalization with increasing age (Glenn et al., 2012a; Michalska et al., 2016; Schiele et al., 2016). Hence, generalization reflects a protective mechanism promoting cautious behavior in childhood, especially in new environments, which decreases with experience, thus leading to a reduction in generalization with advancing age.

It must be pointed out in this context that fear generalization could also have a contrary effect and get maladaptive. At this point, it is important to note, that there is a difference between the terms *fear* and *anxiety*. Whereas fear relates to a known specific source of threat, anxiety follows from a more unpredictable or unknown threat and often lead to generalization of fear to stimuli, which were never associated with threat resulting in diffuse anxiety. If we consider anxiety disorders, exactly this generalization seems to be "the problem". The term *generalized anxiety disorder* (GAD) suggests that this disorder is a matter of diffuse anxieties without any concrete "real" source of threat. In phobias, generalization could play an important role, too. In panic patients with agoraphobia, for example, anxiety of a relevant place could be generalized to other places until than patients are only very hardly able to go outside of their homes and are captured in their apartments. Thus, fear generalization seems to play an important role in the pathogenesis of anxiety disorders (AD) (see e.g. Britton et al., 2013; Dunsmoor & Paz, 2015; Dymond, Dunsmoor, Vervliet, Roche, & Hermons, 2015; Lau et al., 2008; Lissek et al., 2010; Lissek et al., 2014b).

Within mental disorders, AD are the most prevalent (Jacobi et al., 2014; Kessler et al., 2012a). AD are typically characterized by an early onset (Kessler et al., 2007) and persist into adulthood (Beesdo-Baum et al., 2015; Cohen, Cohen, & Brook, 1993) suggesting that it might be important to identify children at risk for AD and permitting premature approaches and treatments to prevent manifest AD in adulthood. The assumption that fear generalization is thought to decrease with increasing age in healthy subjects (see e.g. Glenn et al., 2012a; Michalska et al., 2016) suggests that an early age (e.g. the period during late childhood and early adolescence) may be developmentally critical in understanding developmental aspects of fear learning and generalization, and as a result in understanding the pathogenesis of AD.

Among other factors, early temperamental factors predispose for AD (Chambers, Power, & Durham, 2004). Trait anxiety, for instance, as indexed by the State-Trait Anxiety

Inventory (STAI; Spielberger, Gorsuch, & Lushene, 1970) is a stable personality trait, which was roughly defined as reflecting differences between individuals in the tendency to evaluate situations as threatening, irrespective of real threat and was supposed to be a risk factor for developing AD (Chambers et al., 2004; Spielberger, 1972). Seligman, Ollendick, Langley, and Baldacci (2004) demonstrated that the STAI in children differentiates individuals with AD from individuals without AD. This anxiety-related personality factor is also associated with altered fear learning (Barrett & Armony, 2009). Pilot studies examining associations between trait anxiety levels and *fear conditioning* in adolescents (Kadosh et al., 2015) suggested a mislabelling of safety cues in high anxious (HA) adolescents in contrast to low anxious (LA) adolescents. However, results relating to the correlation of trait anxiety and fear learning are equivocal (Pineles, Vogt, & Orr, 2009).

Since pathological anxiety has high comorbidity rates with other disorders (Kessler, Petukhova, Sampson, Zaslavsky, & Wittchen, 2012b), long-range outcomes for both the health care system and particularly affected patients, and long-term negative consequences for child maturation (Pine, 1997) and hence, for adult life, advancing our understanding of the developmental trajectories of AD is absolutely essential. Therefore, it is important to better investigate and also understand the developmental aspects of fear learning and its generalization, which is a focus of this thesis. A superordinate aim thereby is it to identify psychological and biological processes through which abnormalities in fear conditioning and generalization results in maladaptive behavior (overgeneralization).

1.1 Objectives and Organization of the Thesis

The present dissertation is concerned with developmental aspects of fear learning and fear generalization utilizing both behavioral laboratory-based approaches and functional neuroimaging methods. It is of interest in this thesis to investigate factors, which could influence fear conditioning and generalization in minor participants, e.g. age and sex. In particular, it is focusing on the question if children show fear generalization similar to adults, and if not at which developmental stage children and adolescents cross over to a fear generalization gradient similar to that of adults. Furthermore, this thesis will evaluate if other variables influence fear conditioning and its generalization in underage populations and focus on trait anxiety since anxiety-related personality factors in adults are associated with enhanced condition-ability or impaired extinction of learned fear (Barret & Armony, 2009; Hooker,

Verosky, Miyakawa, Knight, & D'Esposito, 2008). In addition, this work is confronted with the question whether possible age and/or sex differences in fear learning and generalization and also differences according to trait anxiety are reflected in the brain activity during fear learning and generalization. Therefore, for all of the analyses a differential fear conditioning and generalization paradigm was used, which will be explained above (chapter 1.4).

In the first chapter of this thesis, detailed definitions of fear conditioning and fear generalization will be provided. Furthermore, the theoretical background of fear conditioning and fear generalization in underage humans will be summarized. Additionally, fear pathways in the brain and neural correlates involved in fear conditioning and fear generalization will be characterized. Of note, this thesis was restricted to cued conditioning (irrespective of explaining and reviewing existing research on perceptual and non-perceptual fear generalization), whereas contextual conditioning is disregarded here. Aims and hypotheses will be summarized and the paradigm used in all of the following studies in this thesis will be presented. The second chapter will provide two studies conducting to analyze developmental aspects of fear learning and fear generalization. In the third chapter effects of trait anxiety on fear conditioning and fear generalization will be analyzed via behavioral and psycho-physiological measurements. In the fourth chapter, additionally to behavioral data, event-related functional magnetic resonance imaging (fMRI) data during a fear conditioning and generalization task were used to clarify the relationships between brain activity in regions associated with fear learning and generalization and (1) trait anxiety, (2) developmental, and (3) sex effects. In the last chapter, findings will be summarized and discussed with respect to clinical implications.

Due to the fact that this dissertation was developed within a collaborative research center (CRC-TRR-58, project Z02), in which I was merely a part of, I mainly prefer to use the pronoun "we" instead of "I" in this thesis, especially when describing aims and hypotheses as well as methods used in this dissertation.

1.2 Theoretical Context of Fear Learning and Generalization

1.2.1 Fear Conditioning in Underage Populations

Fear conditioning is a central learning mechanism in the pathogenesis of anxiety disorders (Hofmann, Alpers, & Pauli, 2008; Lissek et al., 2005), and thus, fear conditioning is an excellent model to study mechanisms underlying fear learning and memory, and is as such widely used in psychological research. Fear conditioning describes the process whereby a

neutral stimulus becomes a fearful stimulus (or conditioned stimulus: CS), which could elicit a fear response (or conditioned reaction: CR) by repeatedly combined matching with an aversive unconditioned stimulus (UCS) e.g. an electrical shock or a loud tone. The individual learns that the CS is followed by the UCS. Thus, an association is built between memory representation of the CS and the memory representation of the UCS. In differential fear conditioning paradigms individuals learn that a conditioned stimulus (CS+) predicts the UCS, while another stimulus (CS-) is never followed by the UCS and predicts safety (Figure 1.1). The differential fear conditioning paradigm is necessary to validate specificity of learning and rule out non-associative effects.

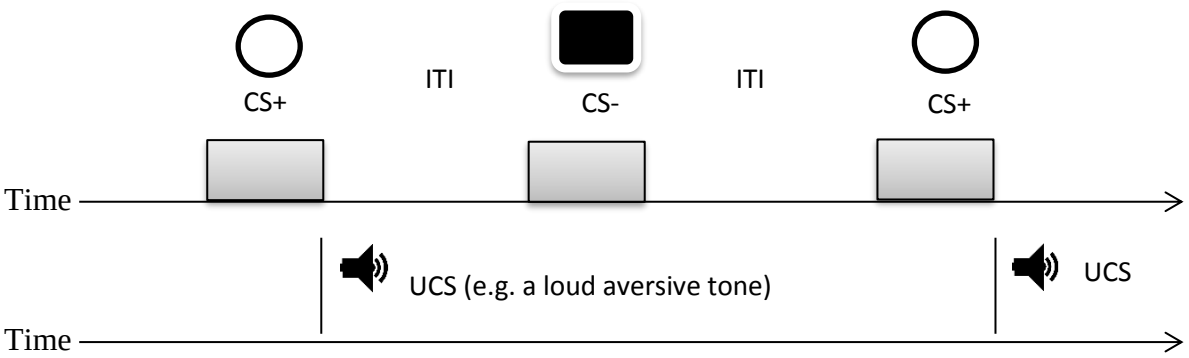


Figure 1.1: Fear conditioning paradigm

In a typical differential fear conditioning paradigm, a stimulus (CS+) is repeatedly combined with an aversive unconditioned stimulus (UCS), while another stimulus (CS-) is never followed by the UCS. The CSs are followed by intertrial intervals (ITI) allowing time for sympathetic arousal to return to baseline.

Conditioned fear responses have been shown in various species, in highly developed species, like humans (e.g. Schiele et al, 2016), just as in ontogenetic prior developed species, like drosophila (Qin & Dubnau, 2010). Thus, there are multiple ways of measuring fear responses ranging from self-report ratings in humans (e.g. Glenn et al., 2012a; Schiele et al., 2016) and psych-physiological responses (e.g. heart rate, skin conductance response; Schiele et al., 2016) or fear reflexes (e.g. startle reflex; Glenn et al., 2012a) to fearful behaviors (e.g. freezing; Meloni, Venkataraman, Donahue, & Carlezon, 2016) and endocrine measurements (e.g. cortisol; Merz, Stark, Vaitl, Tabbert, & Wolf, 2013).

Arousal level and valence self-report ratings are commonly used for measuring emotional states in humans in an opponent way. Arousal represents the fear intension, whereas valence refers to the quality of the responses. Negative valence would occur, e.g. when the stimulation is aversive (e.g. after threat presentation), whereas positive valence should occur after appetitive stimulation (e.g. after safety signals). Skin conductance response (SCR) reflects changes in sweat gland activity that alters the electrical conductivity of the skin, is associated with the sympathetic system, and covaries with the arousal level.

Studies done in children showed that self-report ratings as well as psycho-physiological markers (e.g. SCR) are reliable measurements (e.g. Gao et al, 2010; Haddad, Lissek, Pine, & Lau, 2011; Lau et al., 2008; Morrow, Boring, Kenough, & Haesly, 1969; Pattwell et al., 2012). Most studies, however, used more than one instrument to measure CRs (e.g. Britton et al., 2013; Craske et al., 2008; Glenn et al., 2012a; Lau et al., 2011; Liberman, Lipp, Spence, & March, 2006; Neumann, Waters, Westbury, & Henry, 2008; Waters, Henry, & Neumann, 2009).

Studies investigating subjective (e.g. valence and arousal ratings) and psycho-physiological (e.g. skin conductance response) levels of CR are especially important considering the fact that self-report ratings are dependent on *contingency awareness*. The contingency refers to the probability that a CS will followed by an UCS. In a study of Waters et al (2009), only 55% of non-anxious children reported contingency awareness and in Glenn et al. (2012a), only aware children showed fear potentiated startle to the CS+.

A first famous experiment investigating fear learning in children came from Watson and Rayner (1920). They repeatedly presented a loud aversive tone (UCS) together with a white rat until the usually neutral stimulus (the white rat) came to elicit fear without the UCS. Over 50 years later, an experiment on differential fear learning showed that even infants could learn to differentiate between CS+ and CS- (Ingram & Fitzgerald, 1974). Some studies investigating fear learning in children used a loud tone as UCS and demonstrated that fear learning in children increased with age (e.g. Block Sersen, & Wartis, 1970; Gao et al., 2010). Lau et al. (2008; 2011) used a paradigm with a fearful face and a loud scream as UCS and demonstrated the effectiveness of this paradigm. This "screaming lady" paradigm was shown to be suitable to elicit differential fear responses (Glenn, Liebermann, & Hajcak, 2012b). Glenn et al. (2012a) used this "screaming lady" paradigm in children aged 8 to 13 and showed that younger children were less able to discriminate between threat and safety stimuli than older children. The

increased discrimination-ability with age is associated with developmental patterns of neural activity during fear learning (Lau et al., 2011, see below chapter 1.2.4). Furthermore, a study of Gamwell et al. (2015) with children between 8 and 13 years old suggested that there are early sex differences in fear conditioning pattern with females showing less discrimination between danger and safety signals compared to age-matched males.

Disturbances in fear conditioning seem to contribute to anxiety disorders in humans (Britton et al., 2013; Waters et al., 2009; Lieberman et al., 2006; Lau et al., 2008). However, the very small number of studies comparing the fear conditioned responses of healthy and anxious children showed inconsistent findings. Some studies found increased fear responses in anxious minors to both CS+ and CS- (Britton et al., 2013; Waters et al., 2009; but see Pliszka, Hatch, Borcherding, & Rogeness, 1993), and showed that anxious minors are less able to differentiate the stimuli after acquisition (Liberman et al., 2006) indicating impaired safety signal learning in anxious children. Other studies showed that anxious minors react generally stronger to the CS+ compared to healthy minors (Lau et al., 2008). Several studies examined associations between trait anxiety levels and fear conditioning, mostly in adults but also in adolescents (Kadosh et al., 2015) with inconsistent findings. For instance, some studies reveal that high trait anxiety is associated with deficient safety learning (Gazendam, Kamphuis, & Kindt, 2013; Haaker et al., 2015), whereas other studies found no effect of trait anxiety in adult populations (e.g. Torrents-Rodas et al., 2013).

The various UCSs, the different ways of measurement, and different age ranges may contribute to the fact that findings are inconsistent across studies.

1.2.2 Fear Generalization in Underage Populations

Fear generalization describes a more complex learning process whereby the conditioned fear responses extend to stimuli (generalization stimuli, GSs), which are similar to the CS+ but never followed by the UCS. The generalization gradient usually diminishes as a function of reduced similarity between GSs and CS+ (Ghirlanda & Enquist, 2003). A steep and quadratic versus a shallow and linear gradient indicates limited versus strong generalization, respectively. Commonly, fear generalization gradients rely on several intermediate GSs (Lissek et al., 2010), which are needed to detect subtle generalization effects and are crucial to identify when gradients start to diverge between groups. A comparison of generalization gradients between healthy adults and patients with various anxiety disorders revealed overgeneralization of

conditioned fear, i.e. more shallow and linear generalization gradients, in panic disorder (Lissek et al., 2010), post-traumatic stress disorder (PTSD) (Lissek & Grillon, 2012), and generalized anxiety disorder (GAD) patients (Lissek et al., 2014b; but see Tinoco-González et al., 2015). Additionally, enhanced generalization of conditioned fear has been found to predict anxiety levels in healthy adults six months later (Lenaert et al., 2014). Thus, overgeneralization of conditioned fear seems to be a characteristic of anxiety disorders and likely constitutes a risk factor in their pathogenesis.

In children, however, there is remarkably less research in fear generalization. A study of Glenn et al. (2012a) investigated the generalization of conditioned fear in children (*N*=40, 8-13 years). This study on the basis of fear-potentiated startle and fear rating data found that while all children were able to differentiate between the CS+ and CS- only older children showed a decline in response strength from the CS+ over the GS to the CS- reminiscent of fear generalization patterns in adults. By contrast, younger children were characterized by larger startle responses and fear ratings to both the CS+ and CS- relative to the GS. Animal research using one or more GSs also supports the conclusion of better discrimination with advancing age (Campbell & Haroutunian, 1983; Rudy & Pugh, 1996). Furthermore, Shechner et al. (2015) designed a novel task for examining anxious and healthy children and adults. In this task, a bell was paired with an aversive alarm (UCS) eliciting fear responses. In a further study of this research group, they used this novel paradigm in 5-10-year old children and demonstrated that fear generalization was qualified by child age with better discrimination in older children (Michalska et al., 2016).

1.2.3 Fear Pathways in the Brain

Most of the studies considering the neurobiology of fear learning came from neurophysiological research in nonhuman animals, but there are still some studies done in humans, mostly in adults. It is beyond the scope of this dissertation to describe explicitly the literature, but giving an overview of important and relevant findings is necessary for a better understanding of the neural background involved in human fear learning and generalization (for detailed reviews please see e.g. LeDoux, 2000; Pape & Pare, 2010).

The most familiar structure associated with fear pathways is the amygdala. The amygdala is a limbic structure located in the anterior medial temporal lobe. It is involved in fear learning, fear regulation, and fear memory formation (Hamm & Weike, 2005). Evidence from

animal research has demonstrated that lesions of the amygdala block measures of (un)conditioned fear (e.g. Davis, 2000; Fanselow, 1994; Kapp, Whalen, Suoole, & Pascoe, 1992; LeDoux, 2003). In the opposite way, electrical stimulation of the amygdala provokes fear-typical behavior in many animals, including humans (Gloor, 1992). Accumulating evidence suggests that the amygdala receives extensive afferent projections from a number of brain systems including sensory systems and also higher-order association cortex (e.g. LeDoux, Farb, & Romanski, 1991; McDonald, Mascagni, & Guo, 1996). There is also a direct projection from the thalamus to the amygdala (LeDoux, 2002; Walker & Davis, 2002). Efferent projections from the amygdala are widespread, modulating information processing broadly across the brain. Thereby, individual subnuclei of the amygdala play different functional roles (for a review, see e.g. McDonald, 1998). The nuclei most often implicated in fear conditioning include the basal (B), accessory basal (AB), lateral (La) and central nuclei (Ce). The L, AB, and B can be considered as the basolateral complex (BLA). This complex (BLA) receives sensory information and is therefore important for forming the association between CS and UCS. The region seems to be also involved in consolidation and fear memory (Schafe, Nader, Blair, & LeDoux, 2001). The Ce, on the other hand, receives projections from the BLA and is involved in initiating the CR through output projections to the hypothalamus and brainstem structures.

LeDoux (2002) has shown that fear conditioning can be obtained without any cortical processing of the CS and that direct projections between the thalamus and the amygdala exist, which may be involved in transmitting the information about the CS to the amygdala (LeDoux, Farb, & Ruggiero, 1990). Based on animal evidence, LeDoux (1995) suggested that fear may be processed by two neural pathways that are the thalamo-amygdala pathway and the thalamo-cortico-amygdala pathway. The former is sufficient for the rapid triggering of emotion and for this reason is called the quick-and-dirty pathway or “low road”. The latter involves cortical pathways before reaching the amygdala and is somewhat longer and slower, but the analysis of the emotional stimulus is more complete and thorough. This pathway is called the “high road”. How it results is clear from the denomination of the pathways. The amygdala is the central region, crucial for both “quick-and dirty” and “high-and-cognitive” processing of emotional (fear) inputs. Furthermore, the amygdala plays the central role in the acquisition of fear conditioning.

1.2.4 Neural Correlates of Fear Learning and Fear Generalization

Further relevant brain regions beside the amygdala that are involved in fear learning (Maren, 2001) including the hippocampus, the ventromedial and dorsomedial prefrontal cortex (vmPFC/dmPFC) and the dorsal anterior cingulate cortex (dACC) (for further neural signatures of human fear conditioning please see an extended meta-analysis of fMRI studies e.g. by Fullana et al., 2016). These regions are supposed to mature with increasing age (Monk, 2008). The hippocampus is important for contextual encoding and thus is thought to provide a substrate for binding the features of the context into a unitary representation, and may be specialized for calling to mind a full representation of a feared context from partial information through the process of pattern complementation (O´Reilly & Rudy, 2001). The dACC thickness is associated with enhanced fear acquisition (Milad et al., 2007) and the vmPFC easily spoken is important for inhibitory control over the amygdala, which is especially important in consideration of fear generalization (see e.g. Greenberg, Carlson, Cha, Hajcak, & Mujica-Parodi 2013a). A cross-sectional study investigating children from 4 years of age to adults found that amygdala and the medial PFC were positively connected prior to age 10 years and negatively connected after the age of 10 years indicating a developmental shift in functional connectivity between these brain areas during viewing fearful faces (Gee et al., 2013).

Neuroimaging studies in humans have implicated the amygdala activation during fear conditioning (e.g. Phan, Wagner, Taylor, & Liberzon, 2002). Fear generalization was particularly implicated with amygdala, vmPFC, insula, and ACC (Cha et al., 2014; Greenberg, Carlson, Cha, Hajcak, & Mujica-Parodi, 2013b). The dorsolateral PFC (dlPFC) has been implicated in fear learning through its role in category learning (Miller & Cohen, 2001) and fear regulation (Bishop, Duncan, Brett, & Lawrence, 2004a; Delgado, Nearing, LeDoux, & Phelps, 2008; Goldin, Manber, Hakimi, Canli, & Gross, 2009; Ochsner, Bunge, Gross, & Gabrieli, 2002; Somerville, Whalen, & Kelley, 2010) indicating one important function of dlPFC during fear learning is to demarcate the boundaries separating stimuli into categories of fear-relevant (threat cues) and fear-irrelevant (safety cues) stimuli (Lau et al., 2011).

Results from threat studies of generalization found gradually decreasing activations in anterior insula, dACC and dmPFC, and gradually increasing activations in vmPFC as the GS differentiates from CS+ (Greenberg et al., 2013a; Greenberg et al., 2013b). According to Dunsmoor, Prince, Murty, Kragel, and LaBar (2011), brain activity related to intensity-based fear generalization was localized in the striatum, insula, thalamus, and subgenual cingulate

cortex. According to Lissek, et al. (2014a), such intensity-based fear generalization effects reflect the common effects of the emotional intensity of the GS and the degree to which the GS resembles the CS+. The authors demonstrated that the psychophysiological expression of generalized fear correlated with amygdala activity, and that connectivity between the amygdala and extrastriate visual cortex was correlated with individual differences in trait anxiety.

Lissek and colleagues (2014a) connected animal and human research in conditioning and established a preliminary neural model of conditioned fear generalization, revealing a "positive" generalization gradient (reflected by declines in responding as the presented stimulus differentiates from CS+) i.e. in bilateral insula, and dmPFC, and a "negative" gradient i.e. in vmPFC and bilateral ventral hippocampus (reflected by inclines in responding as the stimulus differentiates from CS+). Functional connectivity analyses, using left and right ventral hippocampus as seed regions, revealed increased coupling with the amygdala and insula during presentation of stimuli resembling the CS+, and increased coupling with the vmPFC during presentation of stimuli with the least resemblance to the CS- (see review of Dymond, et al., 2015; Lissek et al., 2014a). Findings support a neural model of conditioned fear generalization (Lissek, 2012), in which CS+ and GSs in sensory cortex undergo "schematic matching" (Lissek et al., 2014a), or same-different assessment by the hippocampus (Otto & Eichenbaum, 1992; Sander, Grandjean, & Scherer, 2005). However, as Onat and Büchel (2015) demonstrated, fear generalization is not passively driven by perception, but is an active process integrating threat identification and ambiguity-based uncertainty to or initiate a flexible adaptive fear response.

A small number of studies have investigated developmental trends in activity in the above neural structures: Lau et al. (2011) used a by then novel fear conditioning paradigm to compare the behavioral and neural correlates of threat/safety discrimination learning in adolescents and adults using fMRI. They found that compared to adults, adolescents reported less discrimination learning and that adolescents were more likely than adults to engage early-maturing subcortical structures during discrimination learning. Thus, higher responses to CS+ in the amygdala and hippocampus were found in adolescents compared to adults. However, only adults' engagement of late-maturing PFC regions correlated positively with fear ratings during discrimination learning. The findings of Lau et al. (2011) suggest that maturational differences in subcortical and PFC regions between adolescent and adult brains may relate to age-related differences in threat/safety discrimination.

Britton et al. (2013) compared PFC function in anxious and healthy adolescents and adults during fear learning and generalization. They found that relative to their age-matched peer groups, anxious adolescents exhibited a u-shaped pattern of activation in vmPFC with greater activation to the most extreme stimuli, whereas anxious adults exhibited reduced activation when appraising threat indicating that vmPFC dysfunction is age-specific.

Haddad, Bilderbeck, James, and Lau (2015) examined how neural responses to threat and safety cues differ between anxious and non-anxious adolescents (age 12-17), and especially how such differences emerge across development. They found that neural activation to the threat cue correlated positively with age in e.g. the dACC and bilateral dlPFC. Neural activation to the safety cue was modulated differently by age: a more positive association between activation and age was found in the non-anxious group compared to the anxious group in various regions e.g. medial/dorsolateral PFC, anterior insula, and amygdala indicating that maturation of the neural substrates of fear responses to safety cues are perturbed in anxious adolescents (Haddad et al., 2015). Thus, whereas the maturation of neural activation to threat stimuli was similar between high-anxious and low-anxious adolescents, the neural response to safety cues matured differently: a positive association between age and neural activation was observed in the PFC as well as in the amygdala in the low-anxious adolescents but not in the high-anxious volunteers (Haddad et al., 2015). Aberrant brain maturation for both the amygdala and the prefrontal cortex (PFC) has been reported for patients with AD: Reductions in amygdala volume during childhood (Milham et al., 2005) and adolescence (Mueller et al., 2013) were reported in combination with decreased grey matter (GM) volumes in the PFC (Wehry et al., 2015) when comparing AD patients to healthy subjects (Strawn et al., 2013; Strawn et al., 2015). In a study with healthy children aged from 7 to 9 years high childhood anxiety was associated with enlarged amygdala volume (Qin et al., 2014). In addition, both structures have been related to fear conditioning in numerous studies with the PFC reflecting the anticipation of danger by responding differently between safe stimuli that previously predicted danger (CS+) and "naive" safe stimuli. The amygdala, in return, reacted strongest to fear-predictive stimuli (Schiller, Levy, Niv, LeDoux, & Phelps, 2008). Individuals with high trait anxiety show low ventromedial/dorsomedial pre-frontal cortex (vmPFC/dmPFC) activity, but high amygdala activity, when attending to cues that predict threat (Indovina, Robbins, Núñez-Elizalde, Dunn, & Bishop, 2011; Klumpp et al, 2011). Results indicate that individuals with high trait anxiety levels show more fear and were less able to suppress their fear resulting in potentially stronger

fear reactions. Additionally, healthy individuals with higher anxiety demonstrate greater functional connectivity between dmPFC and the amygdala during rest (Kim et al., 2011; Robinson, Charney, Overstreet, Vytal, & Grillon, 2012) indicating that effective connectivity of brain regions supporting emotion processes may vary with negative affect. Additionally, Sehlmeyer et al. (2011) investigated neural correlates of trait anxiety in fear learning and found that high levels of trait anxiety are associated with increased amygdala activation and reduced dACC recruitment, reflecting an increased resistance to extinct fear responses, which may enhance the vulnerability to developing AD.

1.3 Aims and Hypotheses

A superordinate aim of the present dissertation is a contribution to a better understanding of the mechanisms of fear learning. A special focus is on developmental aspects of fear learning and fear generalization. Precise aims are 1) to come to a better understanding of developmental aspects of fear learning and fear generalization, 2) to investigate other influencing aspects on fear learning and fear generalization, e.g. the influence of anxiety traits, and 3) to examine neural correlates during fear learning and fear generalization in underage subjects, especially with respect to trait anxiety, sex, and developmental aspects. Given that neurobiological changes are observed in areas critically involved in the fear neurocircuitry (Whalen, 1998), fear learning paradigms can offer some insights into the mechanisms underlying the neurobiological ontogeny of anxiety (Britton, Lissek, Grillon, Norcross, & Pine, 2011).

We hypothesized that there is a better cue discrimination with advancing age. More precisely, we first predicted heightened fear generalization according to subjective (subjective ratings) and psycho-physiological (SCR) arousal in healthy children (aged 8 to 10 years) as compared to healthy adults. In addition, we further hypothesized better stimulus discrimination in participants at early adolescence (12-year-old participants) as compared to children (8 to 11 years of age): If stimulus discrimination would improve by the age of 12 years, then we would expect to find that arousal to CS+ and CS- would differ significantly stronger in 12-year old children compared to children aged 8 to 11 years. Additionally, we further hypothesized that these developmental differences were displayed in the brain, with stronger activation in frontal regions (associated with fear inhibition) with resemblance to CS+ with increasing age.

Despite age, we also hypothesized that other factors (e.g. sex, contingency awareness, and trait anxiety) influence fear learning and generalization. We assumed that 12- year old boys

are better in discriminating stimuli as compared to girls meaning that differences in arousal to threat cues compared to safety cues would be higher in 12- year-old boys as compared to girls and to younger boys. This effect of sex was assumed to be reflected in the brain with males were assumed to show stronger activation with approximation to CS+ in frontal regions as compared to females. We further assumed that participants, who were considered to be aware of the CS-UCS contingency, would show better stimulus discrimination according to valence and arousal ratings as compared to unaware participants. Relating to trait anxiety, we hypothesized that participants with high trait anxiety would show more fear generalization due to higher fear responses to (ambiguous) safety cues resulting in more linear fear generalization gradients as compared to more quadratic gradients when trait anxiety is low. We further hypothesized a manifestation of heightened trait anxiety in underage participants not only in terms of altered fear learning and generalization but also in terms of impaired fronto-limbic processing. More precisely, we hypothesized that high trait anxiety would result in enhanced fear generalization as reflected by higher activation in fear-related brain regions (e.g. amygdala) with resemblance to CS+ and impaired activation in frontal brain areas, which are associated with fear inhibition.

Four studies were conducted to analyze these hypotheses, starting with 2 experiments analyzing developmental differences. Experiment 1 first compared fear conditioning and fear generalization in children aged 8 to 10 vs. adults. In a further step, fear learning and its generalization in children aged 8 to 12 years were compared, additionally with respect to sex. The second part investigated the influences of trait anxiety in children aged 8 to 12 years. In all of these studies, subjective measures of valence, arousal, and contingency awareness as well as skin conductance response (SCR) were measured. The magnitude of SCR reliably increases during presentations of CS+ making it a good index of conditioned fear (Pattwell et al., 2012; Waters et al., 2009). Finally, we investigated the neural processes of fear conditioning and generalization, and additionally the effects of age, sex, and trait anxiety during fear learning and fear generalization in a subsample of healthy children and adolescents using fMRI.

In all of the studies, I usually referred to the paradigm described in the following section.

1.4 Fear Conditioning and Fear Generalization Paradigm

Across all studies, the same study design was used including the way of recruiting with inclusion and exclusion criteria for the participants, the stimuli, the experimental paradigm and general procedure, the ratings of valence, arousal, and contingency awareness, physiological recordings and data reduction of SCR data, and statistical analysis of behavioral data.

Sample

All participants were recruited from the community and primary/secondary schools in the greater region of Wuerzburg within the context of the collaborative research center CRC-TRR-58 subproject Z02 funded by the German Research Foundation (DFG). For recruiting minors, we first obtained approval from education authorities for addressing the principals of schools for informing them about our research. I informed schools and parents, for instance by parent-teacher conferences, gave flyer and fact sheets of the exact study aim and approach to them and talked to all interested families before I invited them to the study due to inclusion and exclusion criteria. Inclusion criteria were Caucasian descent, right-handedness (Edinburgh Handedness Inventory; Oldfield, 1971), and fluency in German. Exclusion criteria were manifest or lifetime DSM-IV axis I disorder, severe medical conditions, and intake of psychoactive medication. An IQ < 85 as ascertained by the German version of the Culture Fair Intelligence Test 2 (Weiss, 2006) was defined as an additional exclusion criterion for children. In all children, absence of DSM-IV axis I disorder was ascertained using the German versions of the Diagnostic Interview for Mental Disorders for Children and Adolescents (Kinder-DIPS; Schneider, Unnewehr, & Margraf, 2009).

Children had to answer to questionnaires: Trait Anxiety was assessed using the German version of the *Trait scale of the State-Trait Anxiety Inventory for Children (STAIC-T*; Unnewehr, Joormann, Schneider, & Margraf, 1992). The STAIC is a self-report scale to determine the level of trait anxiety on 20 statements on a three-point Likert scale (1) “almost never”, (2) “sometimes” and (3) “often”, resulting in a sum score between 20 and 60. The German *Zürcher Life-Event List* (*ZLEL*; Steinhausen & Winkler-Metzke, 2001) was used to measure negative life events. It consists of 36 items, including dimensions of school, family, peers and potential traumatic events, e.g. accidents, deficits and losses. Questions could be answered either with “yes” or no”. Additionally, if “yes” was the answer the retrospective subjective stress on a 5-point Likert scale from -2 to +2 would be requested. Items rated with

+1 or +2 would be considered as positive life events while items rated with -1 or -2 would be considered as negative life events. Positive and negative life events would be numbered separately. Negative life events > positive life events was set as criterion of the presence of negative life experience (LE).

All of the following studies were approved by the ethical committee of the Medical Faculty of the Julius-Maximilian-University of Würzburg (106/10; 204/15) and complied with the latest version of the declaration of Helsinki. All participants as well as their parents (in the case of minors) gave written informed consent and each family was paid €50 compensation for their participation, and additionally €15 for participating in the fMRI study.

Stimuli

Pictures of two actresses with neutral facial expression (NimStim Face Stimulus Set; Tottenham et al., 2009) served as either the CS+ or CS-, with one of the two faces being randomly selected as the CS+ for each participant. The UCS was a 95-dB female scream (International Affective Digital Sounds system) presented simultaneously with a fearful facial expression of the same actress assigned as the CS+. Four generalization stimuli depicting gradual morphs from CS+ to CS- in 20%-steps (GS1-4) were created using the graphics software Sqirlz Morph Version 2.1 (Xiberpix, Solihull, UK).

Stimulus presentation was controlled using Presentation software version 17.2 (Neurobehavioral Systems, Inc., Albany, CA, USA). CSs and GSs were presented for 6 seconds each. The UCS was presented immediately following CS+ offset for 1.5 seconds. Inter-trial intervals varied from 9-12 seconds, during which a white fixation cross was displayed centrally on the screen. Stimulus order was pseudo-randomized so that the same stimulus could not appear more than twice in a row.

Task

A schematic representation of the paradigm was demonstrated in Figure 1.2 (cf. Schiele et al., 2016). The paradigm was based on Lau et al. (2008). Some studies indicate that the "screaming lady" paradigm represents a more powerful UCS than other UCSs employed in research with children and adolescents (Lau et el., 2008; Lau et el., 2011; Schmitz et al., 2011;). The experiment was divided into three consecutive phases: pre-acquisition, acquisition, and generalization. Pre-acquisition consisted of 4 CS+ and 4 CS-; no UCS was presented. During

acquisition, 12 CS+ and 12 CS- were presented. The CS+ was paired with the UCS on 10 trials. The generalization phase consisted of 12 CS+, 12 CS-, and 12 of each of the four GSs. Half the CS+ trials were followed by the UCS to prevent premature extinction. CS- and all GSs were never paired with the UCS. Participants were not informed of the CS-UCS contingencies. Acquisition and generalization trials were separated into two phases, each containing half the trials per phase, i.e. 6 presentations per stimulus category.

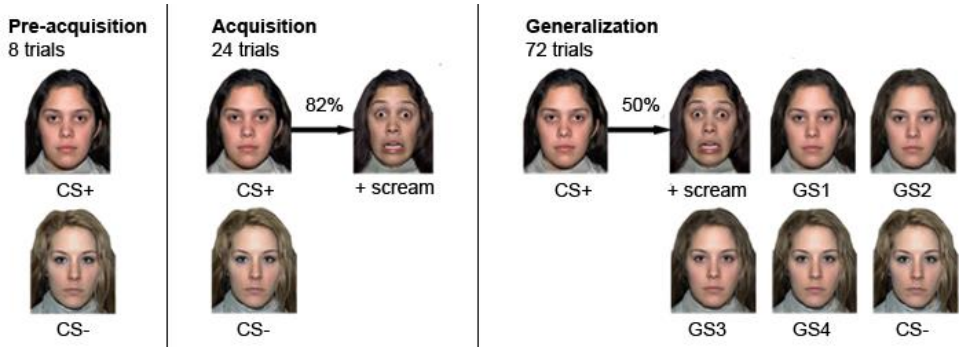


Figure 1.2: Schematic representation of the fear conditioning and fear generalization paradigm (cf. Schiele et al., 2016; pictures of the actresses were used from NimStim Face Stimulus Set; Tottenham et al., 2009).

Participants were instructed to passively view pictures of two female faces, and that an unpleasant sound would be heard occasionally. They were told that it would be possible to become startled and/or frightened and that participation could be discontinued at any time.

Subsequent, an extinction session, in which 18 CS+ and 18 CS- without UCS were presented, was executed for ethical reasons to ensure that no permanent conditioning would occur.

Ratings

Following (pre)-acquisition and generalization, participants rated each stimulus on arousal, valence, and UCS expectancy. Arousal and valence were indicated on 9-point Likert scales, ranging from "very calm" (1) to "very arousing" (9), and "very unpleasant" (1) to "very pleasant" (9), respectively. UCS expectancy was recorded in percent on a scale from 1-100 in 10% increments as the probability of an aversive noise following each stimulus.

Contingency Awareness

Participants were considered aware of the CS-UCS relationship, if UCS expectancy ratings were higher for the CS+ than the CS- and, additionally in the first experiment (in which adults also participated), if UCS expectancy for the CS- was no higher than 50%. Contingency awareness after acquisition and generalization was determined using the ratings after the second acquisition and generalization phase, respectively.

Physiological Recordings and Data Reduction

Throughout the experiments, skin conductance responses (SCRs) were recorded continuously using Brainproducts V-Amp-16 and Vision Recorder software (Brainproducts, Gilching, Germany) at a sampling rate of 1000Hz and analyzed offline using Vision Analyzer 2 software (Brainproducts, Gilching, Germany; cf. Schiele et al., 2016). SCR was recorded from the thenar and hypothenar eminences of the left hand using two Ag/AgCl electrodes. The amplifier delivered a constant current of 0.5V. The SCR signal was filtered offline with a high cutoff filter of 1Hz and a notch filter of 50Hz. SCR was defined as the base-to-peak difference (in µS) between response onset (900–4000 ms after stimulus onset) and peak (2000–6000 ms after stimulus onset). A minimum response criterion of 0.02µS was applied, with lower responses scored as 0. SCR data was normalized following an approach described by Dunsmoor and colleagues (2011), i.e. by computing generalization gradients for each phase and block as a function of the response to one stimulus type relative to the sum of responses to all stimuli. That is, for each of the pre-acquisition, acquisition and generalization phases, the sum of SCRs to each stimulus was divided by the sum of responses to all stimuli, resulting in an index for each stimulus type that allows for the direct comparison of generalization patterns between groups.

Statistical Analyses

All statistical tests were carried out using SPSS version 23 (SPSS Inc., Chicago, Illinois, USA). Ratings were analyzed using repeated-measures ANOVAs with the between-subject factor *group* (study 1.1: adults vs. children; study 1.2: age (8-12); study 2: STAIC score; study 3: STAIC group LA vs. HA) and the within-subject factor *stimulus type* (CS+/CS- at acquisition, CS+/GS-/GS1-4 at generalization). For analysis of acquisition and generalization blocks, two additional factors were included: the within-subject factor *phase* (acquisition and

generalization phase1 vs. phase2) to detect possible reaction changes between first and second phase, and the between-subject factor *awareness* (unaware, aware), since awareness of the CS-UCS relationship may influence the conditioned responses (Lovibond & Shanks, 2002). Sex was set as confounding variable in analyses within children.

ANOVAs were followed by post-hoc t-tests where necessary. Alpha was set at .05 and Bonferroni correction was applied where necessary. Greenhouse-Geisser corrections for non-sphericity were performed where indicated, though uncorrected degrees of freedom are reported for the sake of better readability. Corrected *p*-values, and partial η^2 are reported.

2. Developmental aspects of fear learning and fear generalization

2.1 Developmental aspects of fear: Comparing the acquisition and generalization of conditioned fear in children (aged 8 to 10 years) and adults

There is a considerable knowledge gap concerning developmental effects on fear conditioning and generalization. Direct comparisons between child and adult populations in particular are necessary to identify developmental vulnerability markers of fear and anxiety. Different paradigms, varying experimental conditions, and small sample sizes make it difficult to generalize from previous findings and limit the ability to compare fear learning across different ages. In the present study, we aim to address this gap in the literature by comparing large samples of healthy children and adults using the same paradigm under the same experimental conditions in order to elucidate age-related differences in the acquisition and generalization of conditioned fear. To this end, all participants were exposed to the differential fear conditioning paradigm followed by the generalization test as described in the above section, measuring subjective (valence and arousal ratings) and psycho-physiological (SCR) indicators of fear learning. While we expected both age groups to show differential conditioning, we predicted heightened fear generalization in children.

Data presented in this chapter have been published as a research article in the journal *Developmental Psychobiology* (Schiele et al., 2016).

2.1.1 Methods

2.1.1.1 Participants

A total of 285 adults and 267 children were recruited from the community and primary schools, respectively. Inclusion and exclusion criteria were as described above. Additional exclusion criteria for adults were excessive consumption of alcohol (>15 units/week), nicotine (>20 cigarettes/day), caffeine (>4 cups/day), drug use, and pregnancy. Absence of DSM-IV axis I disorder in adults was ascertained using the German versions of the Mini International Psychiatric Interview (Sheehan et al., 1998). Seven adults and 19 children were excluded from analysis due to technical errors with physiological recordings. Nine children did not complete the experiment, thus resulting in a final sample of 278 adults (189 female) between the ages of 18 and 50 (mean age: 25.56, *SD*: 6.193) and 239 children (119 female) between 8 and 10 years (mean age: 9.00, *SD*: .812).

2.1.1.2 Statistical Analyses

As already described in chapter 1.4, data were analyzed using repeated-measures ANOVAs with the between-subject factor *group* (adults vs. children) and the within-subject factor *stimulus type* (CS+/CS- at acquisition, CS+/GS-/GS1-4 at generalization), and the two additional factors *phase* and *awareness*. Since analyses did not reveal any effect of awareness on SCR, awareness was omitted as a group factor for the reported analysis of SCR data. For the other dependent variables (valence and arousal ratings), awareness effects were found; however, no awareness x group interactions were found. Therefore, we report awareness effects in a separate section. Finally, we repeated the main analyses in aware participants only, mainly to reveal that the group effects observed in the complete samples are similar in the subsamples of aware participants. Changes in contingency awareness between acquisition and generalization were tested using Chi square (χ^2) tests, and phi coefficients (ϕ) are reported as measures of the corresponding effect sizes.

2.1.2 Results

2.1.2.1 Contingency Awareness

After acquisition, 247 (89%) of the 278 adults and 114 (48%) of the 239 children were considered aware of the CS-UCS relationship. At generalization, 263 adults (95%) and 145 children (61%) fulfilled criteria for contingency awareness. Awareness was higher in adults compared to children both at acquisition (χ^2 (1, N = 517) = 103.29, p < .001, ϕ = -.45) and generalization (χ^2 (1, N = 517) = 88.95, p < .001, ϕ = -.42). In both groups, awareness increased from acquisition to generalization (χ^2_{Adults} (1, N = 278) = 61.88, p < .001, ϕ = .47; χ^2_{Children} (1, N = 239) = 13.46, p < .001, ϕ = .24). The criteria for awareness both at acquisition and generalization were fulfilled by 243 adults, 11 remained unaware throughout the experiment and 20 adults became aware during generalization only. In children, 83 were aware of the stimulus contingency at both assessment times, 63 children remained unaware throughout the experiment and 62 became aware at generalization only. Unexpectedly, four adults and 31 children were aware at acquisition but became unaware at generalization. Since we continued CS+ reinforcement during generalization to prevent premature extinction effects we suspect that this unexpected loss in awareness was due to inattentiveness or inadvertence (e.g. typing mistakes) and reflects unsystematic errors. Therefore, these participants were excluded from all analyses.

2.1.2.2 (Pre-)Acquisition and Generalization Effects

Results for valence and arousal ratings as well as SCR at pre-acquisition and acquisition are depicted in Figure 2.1.1 A-F. Differences between children and adults regarding generalization are depicted in Figure 2.1.2 A–C.

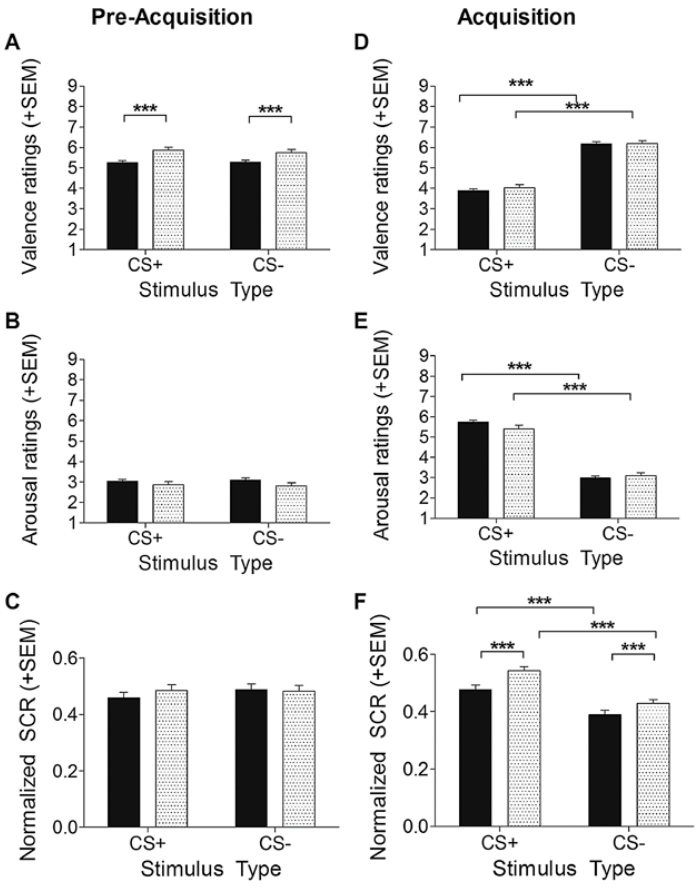


Figure 2.1.1: Children (grey bar) and adults (black bar) displayed robust differential conditioning to the CS+ in valence ratings (top), arousal ratings (center), and normalized skin conductance response (bottom) at acquisition (D–F), but not at pre-acquisition (A–C). ***p < .001; **p < .01; *p < .05.

Pre-acquisition: As depicted in Figure 2.1.1 (A-C), no differences were found between the CS+ and the CS- prior to conditioning in both children and adults for valence and arousal ratings (*p*s ≥ .410) and for SCR (*p*s ≥ .451). However, children rated both the CS+ and the CS- as more pleasant than adults prior to conditioning ($F(1,480) = 15.26$, $p < .001$, $\eta^2 = .03$).

Acquisition: Valence ratings (Figure 2.1.1 D) revealed that after conditioning, both groups rated the CS+ as more unpleasant than the CS-, as indicated by a significant main effect of stimulus type ($F(1,478) = 200.45$, $p < .001$, $\eta^2 = .30$) and a non-significant interaction of stimulus type x group ($p = .138$). Additionally, ratings differed according to phase (main effect phase: $F(1,478) = 4.93$, $p = .027$, $\eta^2 = .01$; interaction stimulus type x phase: $F(1,478) = 5.41$, $p = .020$, $\eta^2 = .01$), with more pleasant valence ratings to the CS- in phase 2 than in phase 1 ($M_{CS-\_Phase1} = 6.04$, $SD_{CS-\_Phase1} = 1.93$ vs. $M_{CS-\_Phase2} = 6.34$, $SD_{CS-\_Phase2} = 2.02$; $t(481) = -3.34$, $p = .001$).

Arousal ratings (Figure 2.1.1 E) were higher for the CS+ relative to the CS- in both groups ($F(1,478) = 252.33$, $p < .001$, $\eta^2 = .35$). Again, no stimulus type x group interaction was observed ($p = .627$).

For SCR (Figure 2.1.1 F), a significant main effect of stimulus type ($F(1,480) = 35.34$, $p < .001$, $\eta^2 = .07$) was observed, with higher SCR to the CS+ than the CS-, but no stimulus type x group interaction ($p = .384$). Additionally, a significant main effect of group was observed ($F(1,480) = 15.77$, $p < .001$, $\eta^2 = .03$), reflecting overall higher SCR in children ($M = .49$, $SD = .06$) than in adults ($M = .45$, $SD = .13$).

Generalization: With regard to valence (Figure 2.1.2 A), a significant main effect of stimulus type ($F(5,2390) = 46.48$, $p < .001$, $\eta^2 = .09$) was observed. No interactions involving the between-subject factor group reached significance (*p*s ≥ .084).

For arousal ratings (Figure 2.1.2 B), a significant main effect of stimulus type ($F(5,2390) = 60.47$, $p < .001$, $\eta^2 = .11$) and a significant interaction of stimulus type x group ($F(5,2390) = 4.10$, $p = .001$, $\eta^2 = .01$) were observed, with differences in the quadratic and cubic components between groups (stimulus type x group quadratic trend: $F(1,478) = 10.38$, $p = .001$, $\eta^2 = .02$; stimulus type x group cubic trend: $F(1,478) = 5.86$, $p = .016$, $\eta2 = .01$). Within-group follow-up tests revealed linear ($F(1,272) = 51.16$, $p < .001$, $\eta2 = .16$) and quadratic ($F(1,272) = 29.74$, $p < .001$, $\eta2 = .10$) trends in adults, and linear ($F(1,206) = 78.12$, $p < .001$,

η2 = .28), quadratic (F(1,206) = 13.12, p < .001, η2 = .06), and cubic (F(1,206) = 3.91, p = .049, η2 = .02) trends in children. Between-group follow-up tests indicated that adults rated the CS+ (t(354) = 3.33, p = .001) as more arousing than children, whereas children rated the GS2 (t(370) = -2.80, p = .005), GS4 (t (370) = -2.57, p =. 010), and CS- (t(377) = -3.53, p < .001) as more arousing than adults. Group differences regarding GS3 ratings (p = .012 uncorrected) did not survive correction for multiple testing, and were not significant for GS1 ratings (t(375) = .38, p = .707).

For SCR (Figure 2.1.2 C), there was a significant main effect of stimulus type (F(5,2400) = 9.40, p < .001, η^2 = .02). A significant interaction of stimulus type x group indicated that generalization gradients differed between groups (F(5,2400) = 2.98, p = .011, η^2 = .01), with a stimulus type x group quadratic interaction (F(1,480) = 6.74, p = .010, η^2 = .01). Within-group follow-up tests revealed a quadratic trend in adults (F(1,273) = 7.07, p = .008, η2 = .03) but not in children (p = .900). Between-group follow-up tests revealed that children were characterized by higher SCR to the GS2 (t(455) = -2.75, p = .006) and GS3 (t(480) = -3.84, p < .001) compared to adults. There were no significant differences regarding CS+, GS1, GS4 or CS- (ps ≥ .53). Again, a main effect of group emerged between adults and children (F(1,480) = 9.74, p = .002, η2 = .02), with higher SCRs in children (M =.17, SD = .02) than adults (M = .16, SD = .03).

In sum, all dependent measures indicate successful conditioning effects in both adults and children, but greater generalization of arousal ratings and SCRs in children compared to adults.

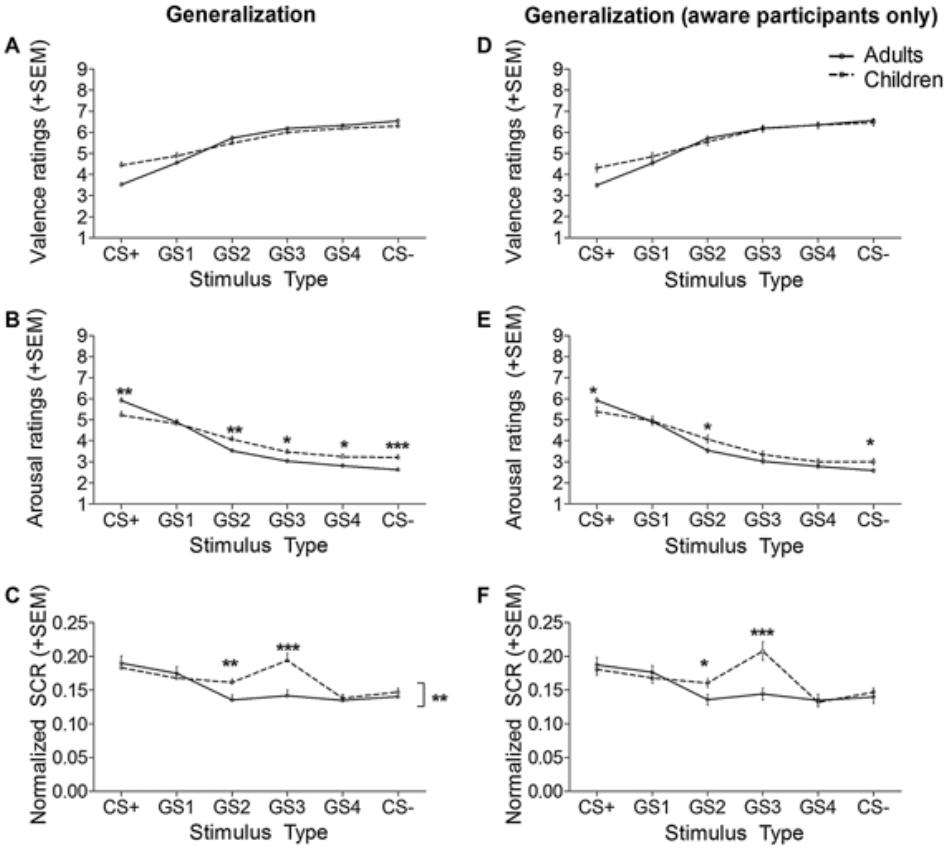


Figure 2.1.2: Greater fear generalization of arousal ratings and SCR was observed in children compared to adults (A–C). The observed generalization differences remained when only participants explicitly aware of the CS-UCS relationship were compared (263 adults, 145 children; D–F). Thus, group differences cannot be explained by the greater proportion of unaware children relative to adults. ***p < .001; **p < .01; *p < .05, #p < .10.

2.1.2.3 Effects of Awareness on Acquisition and Generalization

The above analyses, except for SCR, included the between-subject factor awareness (see Methods), but results concerning awareness are reported here for the sake of clarity since no awareness by group effects were found.

Acquisition: Valence ratings were affected by awareness ($F(1,478) = 14.30$, $p < .001$, $\eta^2 = .03$), with aware participants (children and adults) rating the CS+ as more unpleasant than unaware participants ($M_{Aware} = 3.74$, $SD_{Aware} = 1.69$; $M_{Unaware} = 4.40$, $SD_{Unaware} = 2.18$). For arousal ratings, significant interactions of stimulus type x awareness ($F(1,478) = 12.41$,

$p < .001$, $\eta 2 = .03$) and stimulus type x awareness x phase ($F(1,478) = 7.49$, $p = .006$, $\eta 2 = .02$) were observed. Aware participants rated the CS+ as more arousing in both phase 1 ($t(246) = -2.22$, $p = .027$) and phase 2 ($t(256) = -3.15$, $p = .002$) compared to unaware participants. Awareness differed regarding the CS- in phase 2, with lower arousal ratings in aware compared to unaware participants ($t(251) = 2.66$, $p = .008$) but not in phase 1 ($p = .99$).

Generalization: A significant interaction of stimulus type x awareness was observed for valence ratings ($F(5,2390) = 4.40$, $p = .005$, $\eta 2 = .01$), with more negative ratings of the CS+ ($t(93) = 3.13$, $p = .002$) and in turn a more positive evaluation of the GS3 (t $(480) = -2.61$, $p = .009$) in aware compared to unaware participants, but not for the GS1, GS2, GS4, or CS- ($ps \geq .022$). Arousal generalization gradients differed as a function of awareness and phase. Two two-way interactions (stimulus type x awareness: $F(5,2390) = 6.86$, $p < .001$, $\eta 2 = .01$ and stimulus type x phase: $F(1,478) = 9.45$, $p = .002$, $\eta 2 = .02$) were qualified by a three-way-interaction of stimulus type x phase x awareness ($F(1,478) = 8.25$, $p = .004$, $\eta 2 = .02$). Aware participants were characterized by steeper generalization gradients, indicating better discrimination learning: In both phases, arousal ratings for the CS+ were higher in aware than unaware participants ($t_{Phase1}(94) = -2.67$, $p = .009$; $t_{Phase2}(480) = -2.14$, $p = .033$). GS4 ratings were lower in aware participants in both phases ($t_{Phase1}(89) = 2.95$, $p = .004$; $t_{Phase2}(90) = 3.49$, $p = .001$). GS3 and CS- ratings were affected by awareness in phase 2 only, with lower ratings in aware participants ($t_{GS3}(92) = 3.16$, $p = .002$; $t_{CS-}(92) = 4.29$, $p < .001$).

Taken together, these results indicate that, regarding ratings, aware participants exhibited greater acquisition effects and steeper generalization gradients, however and most importantly, groups did not differ in these awareness effects.

2.1.2.4 Acquisition and Generalization Effects in Aware Participants

Since significantly more adults than children were aware of the CS-UCS contingencies and since awareness modulated the steepness of the generalization gradients, it might be speculated that the observed stronger generalization effects in children were due to the high proportion of unaware children. Therefore, we repeated the above analyses by including only participants, who were aware at generalization (263 adults and 145 children). Importantly, analyses in aware participants only yielded similar results as in the whole sample: Analysis of

arousal ratings (Figure 2.1.2 E) again returned a significant interaction of stimulus type x group, ($F(5,406) = 7.22$, $p < .001$, $\eta^2 = .02$), with differences in the linear, quadratic, and cubic components between groups (stimulus type x group linear trend: $F(1,406) = 7.45$, $p = .007$, $\eta^2 = .02$; stimulus type x group quadratic trend: $F(1,406) = 10.01$, $p = .002$, $\eta^2 = .02$; stimulus type x group cubic trend: $F(1,406) = 8.124$, $p = .005$, $\eta^2 = .02$). Within-group follow-up tests again yielded linear ($F(1,262) = 559.54$, $p < .001$, $\eta^2 = .68$) and quadratic ($F(1,262) = 122.01$, $p < .001$, $\eta^2 = .32$) trends in adults, as well as linear ($F(1,144) = 108.68$, $p < .001$, $\eta^2 = .43$), quadratic ($F(1,144) = 15.61$, $p < .001$, $\eta^2 = .10$), and cubic ($F(1,144) = 9.68$, $p = .002$, $\eta^2 = .06$) trends in children.

Likewise, the observed stimulus x group interaction for SCR data (Figure 2.1.2 F) also remained significant ($F(5,406) = 3.17$, $p = .008$, $\eta^2 = .01$), again with a stimulus type x group quadratic interaction ($F(1,406) = 6.23$, $p = .013$, $\eta^2 = .02$), as well as the main effect of group ($F(1,406) = 8.20$, $p = .004$, $\eta^2 = .02$). Within-group follow-ups confirmed the previous findings with a quadratic trend in adults ($F(1,262) = 15.83$, $p < .001$, $\eta^2 = .02$) but not in children ($p = .133$).

In sum, the stronger generalization effects we observed in children relative to adults in the complete samples were also observable in the subsamples of aware participants.

2.2 The development of fear learning and generalization in children aged 8 to 12 years

Since we found differences in fear generalization between adults and children (aged 8 to 10 years), the question emerged at which time point fear generalization gradients of children and adolescents assimilate to that of adults. Because anxiety disorders typically develop during childhood (Kessler et al., 2007), we investigated a sample of underaged participants aged 8 to 12 years for better understanding developmental changes in fear learning and generalization.

The first aim of this analysis was to investigate fear learning and generalization in a large sample of healthy children for replicating and expanding our results of study 1.1. A second aim was to investigate developmental changes in fear generalization from ages 8 to 12. This aim builds on results of Glenn et al. (2012a) showing age differences in children aged 8 to 13 years, but only with one GS, and study 1.1 showing differences in fear generalization between adults and children (aged 8 to 10 years). Third, because of manifest anxiety disorders are more often in women than in men, we wondered whether there are early sex differences between boys and girls in fear learning and generalization. Findings could help to better understand the developmental trajectories of fear learning and its generalization.

2.2.1 Methods

2.2.1.1 Participants

In the greater radius of Wuerzburg we recruited 473 children (240 female; mean age: 9.7; *SD*: 1.29) children aged 8 to 12 years. Six children canceled the experiment at acquisition, and additionally 12 children canceled at generalization. Additionally, 49 children were excluded from analysis due to technical errors with physiological recordings. Thus, a total of 396 children (199 female; mean age: 9.8; *SD*: 1.3) were included in the study. Descriptive characteristics are given in Table 2.2.1. There were no significant differences between the age-groups neither according to sex (p = .065) nor contingency awareness at acquisition (p = .483) and generalization (p = .291).

2.2.1.2 Statistical Analyses

As already described in chapter 1.4, data were analyzed using repeated-measures ANOVAs with the between-subject factor *age*, the within-subject factor *stimulus type* (CS+/CS- at acquisition, CS+/GS-/GS1-4 at generalization), and the two additional factors

phase and *awareness*. Since analyses did not reveal any Awareness x Age interactions, effects of awareness were reported in a separate section. Additionally, to assess the association between subjective and psycho-physiological arousal to threat and (ambiguous) safety cues and age after controlling for awareness we conducted stepwise regression analyses separately for males and females with responses to late CS+ and CS- as predictor variables and age as the dependent variable (cf. Jovanovic, et al., 2014). The first regression used fear conditioning variables during acquisition as predictors of age. We entered awareness in the first step, arousal to the CS+ in the second step, arousal to CS- in the third step, SCR to CS+ in the fourth step, and SCR to CS- in the final step, doing the same procedure in a second stepwise regression with CS+/CS- during generalization. Changes in contingency awareness between acquisition and generalization and differences between girls and boys were tested using Chi square (χ^2) tests, and phi coefficients (ϕ) are reported as measures of the corresponding effect sizes.

2.2.2 Results

2.2.2.1 Contingency Awareness

After acquisition 211 (53%) participants were considered aware, whereas after generalization 284 (71%) were considered aware of the CS-UCS relationship. Thus, awareness increased from acquisition to generalization (χ^2 (1, N = 396) = 50.19, $p < .001$, $\phi = .36$). After generalization, significantly more girls than boys were aware (χ^2 (1, N = 396) = -5.27, $p = .014$, $\phi = .12$).

Table 2.2.1: Descriptive Characteristics of the sample differentiated by age-groups

Age	N Total	Female (%)	Awareness Acquisition (%)	Awareness Generalization (%)
8 years	84	52	50	68
9 years	80	54	50	78
10 years	106	43	51	76
11 years	74	45	57	65
12 years	52	65	64	69

2.2.2.2 (Pre-)acquisition and Generalization Effects

Results for valence and arousal ratings as well as SCR at acquisition are depicted in Figure 2.2.1 A-C.

Pre-acquisition: The ANOVAs on valence ratings and physiological arousal (SCR) revealed no significant stimulus type effects (*p*s ≥ .184), indicating that the CS+ and the CS- were comparable prior to conditioning independent of age.

However, in arousal ratings sex played a crucial role, with a significant main effect of sex ($F(1,390) = 4.43$, $p = .036$, $\eta^2 = .01$) and a Stimulus Type x Sex interaction effect ($F(1,390) = 6.83$, $p = .009$, $\eta^2 = .02$) indicating higher arousal ratings to CS+ in girls compared to boys ($t(380) = -3.35$, $p = .001$; $M_{girls} = 3.34$, $SD_{girls} = 2.31$; $M_{boys} = 2.63$, $SD_{boys} = 1.88$).

In SCR, there was a significant main effect of age ($F(4,390) = 4.99$, $p = .001$, $\eta^2 = .05$) indicating generally reduced SCR with increasing age.

Acquisition: Valence ratings confirmed the expected conditioning effect as the CS+ was rated as more unpleasant than the CS- (main stimulus type effect: $F(1,385) = 21.01$, $p < .001$, $\eta^2 = .05$; Figure 2.2.1 A). A significant interaction of Stimulus Type x Age reached significance ($F(4,385) = 3.46$, $p = .009$, $\eta^2 = .04$) indicating higher valence ratings to CS+ in older children. However, post-hoc comparisons did not resist Bonferroni correction.

Arousal ratings also indicated successful conditioning as the CS+ relative to the CS- after conditioning elicited enhanced arousal across all age-groups (main stimulus type effect: $F(1,385) = 6.83$, $p = .009$, $\eta^2 = .02$; Figure 2.2.1 B). As at pre-acquisition, sex played a crucial role, with a significant main effect of sex ($F(1,385) = 6.32$, $p = .012$, $\eta^2 = .02$) and a Stimulus Type x Sex interaction ($F(1,385) = 4.50$, $p = .035$, $\eta^2 = .01$) indicating significant higher arousal ratings to CS+ in girls compared to boys ($M_{girls} = 5.71$, $SD_{girls} = 2.37$; $M_{boys} = 4.96$, $SD_{boys} = 2.49$). This was true for phase 1 (CS+: $t(394) = 2.38$, $p = .018$) and phase 2 (CS+: $t(394) = 3.24$, $p = .001$).

SCR also confirmed successful conditioning as CS+ relative to the CS- after conditioning elicited enhanced SCR across all age-groups (main stimulus type effect: $F(1,385) = 4.70$, $p = .031$, $\eta^2 = .01$; Figure 2.2.1 C). As at pre-acquisition, age yielded a significant main effect ($F(1,385) = 11.66$, $p < .001$, $\eta^2 = .11$) indicating again reduced SCR with increasing age. There were differences in SCR between the phases indicating higher SCR in phase 1 (significant main effect of phase: $F(1,385) = 10.66$, $p = .001$, $\eta^2 = .03$).

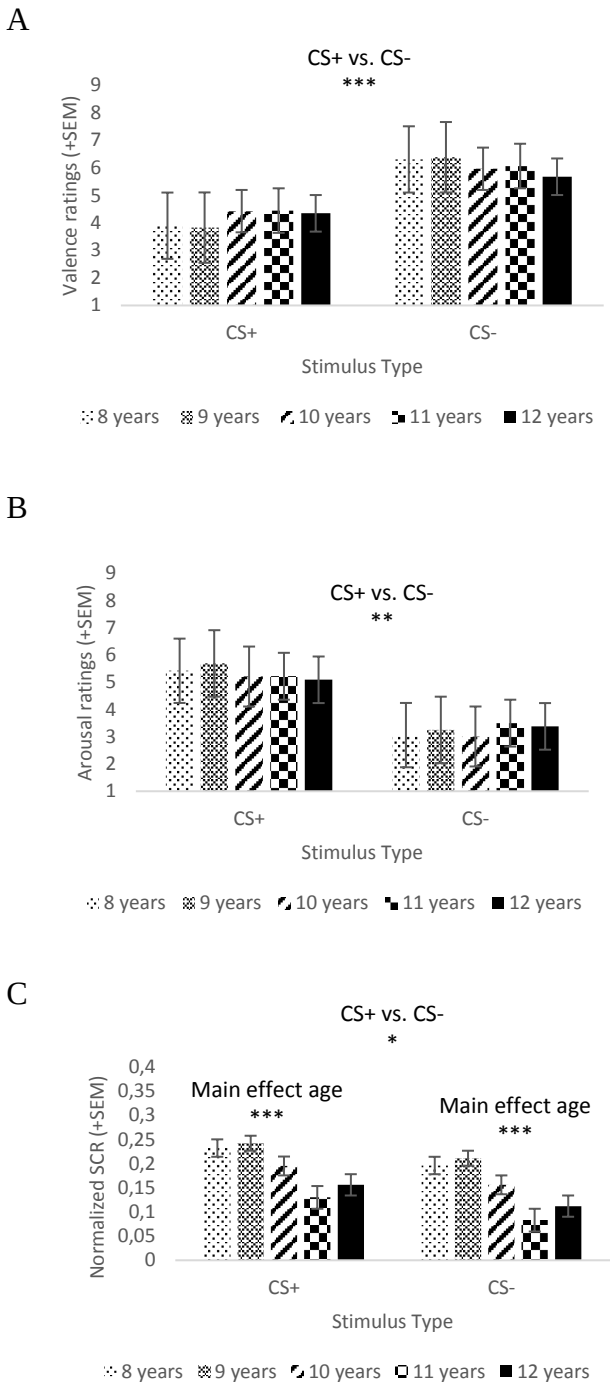


Figure 2.2.1: Children at all age-groups displayed robust differential conditioning to the CS+ in valence ratings (top), arousal ratings (center), and normalized Skin conductance response (SCR) (bottom) at acquisition (A-C). Reduced SCR was found with increasing age. ***p < .001; **p < .01; *p < .05.

In sum, all dependent measures indicate successful conditioning effects in children aged 8 to 12 years.

Generalization: With regard to valence ratings, a significant main effect of stimulus type ($F(5,1925) = 6.58$, $p < .001$, $\eta^2 = .02$) indicated an upward generalization gradient in valence ratings from CS+ to CS-. No interactions involving the between-subject factor age-group reached significance ($ps \geq .306$).

For arousal ratings, a significant main effect of stimulus type ($F(5,1925) = 4.56$, $p = .003$, $\eta^2 = .01$) indicated a downward generalization gradient in arousal ratings from CS+ to CS-. A main effect of sex ($F(1,385) = 6.05$, $p = .014$, $\eta^2 = .02$) suggested, as already after conditioning, that girls show higher arousal ratings compared to boys. A Stimulus Type x Phase x Age interaction ($F(20,1925) = 1.88$, $p = .012$, $\eta2 = .02$) with a Stimulus Type x Phase x Age linear trend ($F(4,385) = 2.44$, $p = .047$, $\eta^2 = .03$) was found. Within-group follow-up tests revealed a linear trend in 10- years-old ($F(1,103) = 5.04$, $p = .027$, $\eta2 = .05$) and 11-years-old children ($F(1,71) = 4.22$, $p = .044$, $\eta2 = .06$), but a quadratic trend in 12-years-old children ($F(1,49) = 5.77$, $p = .02$, $\eta2 = .11$). Age-group differences, however, did not survive correction for multiple testing.

With regard to SCR, a significant main effect of stimulus type ($F(5,1925) = 6.08$, $p < .001$, $\eta^2 = .02$) indicated a downward generalization gradient in SCR from CS+ to CS-. Age played a significant role with a main effect of age ($F(1,385) = 3.51$, $p = .008$, $\eta^2 = .04$) and a Stimulus Type x Age interaction ($F(20,1925) = 4.42$, $p < .001$, $\eta^2 = .04$) with a Stimulus type x Age quadratic trend ($F(4,385) = 4.10$, $p = .003$, $\eta^2 = .04$). Between-group follow-up tests indicated that there were differences in SCR between the age-groups to **CS+** ($F(4,395) = 4.04$, $p = .003$), **GS1** ($F(4,395) = 9.48$, $p < .001$), **GS3** ($F(4,395) = 8.03$, $p < .001$), **GS4** ($F(4,395) = 2.54$, $p = .039$), and **CS-** ($F(4,395) = 7.98$, $p < .001$). Differences relating to GS4, however, did not survived Bonferroni correction. No differences in GS2 were found according to age. Detailed p-values relating to age differences at the stimuli were given in Table 2.2.2.

Analyses in aware participants only yielded similar results: the observed Stimulus x Age interaction for SCR data remained significant ($F(20,1390) = 2.62$, $p < .001$, $\eta2 = .04$).

Table 2.2.2: Significant p-values of age differences in SCRs during generalization

Age differences	CS+	GS1	GS2	GS3	GS4	CS-
8 vs. 12 years	n.s.	**.006**		**.006**		**.010**
8 vs. 11years	**.015**	**.001**		**.001**		**.001**
8 vs. 10 years	n.s.	n.s.		n.s.		n.s.
8 vs. 9 years	n.s.	n.s.	all	n.s.	all	n.s.
9 vs. 12 years	n.s.	**.001**	n.s.	**.003**	n.s.	n.s.
9 vs. 11years	**.003**	**.001**		**.001**		**.001**
9 vs. 10 years	n.s.	**.031**		n.s.		n.s.
10 vs. 12 years	n.s.	n.s.		n.s.		n.s.
10 vs. 11 years	n.s.	n.s.		n.s.		n.s.
11 vs. 12 years	n.s.	n.s.		n.s.		n.s.

Note: Only significant p-values for CS+, CS-, and the generalization stimuli (GS1-GS4) are mentioned and listed in bold letters, whereas not significant p-values are listed as n.s. (not significant). Results indicate that age differences according to SCR are mostly due to differences between 8/9-years-old children and 11/12-years-old children.

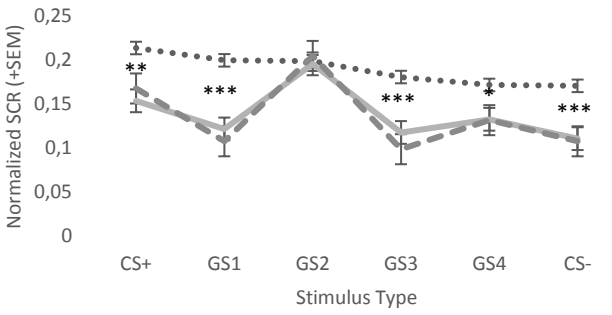
Due to better illustration of age differences and due to differences in trend-analyses with quadratic trends only in 12-years-old children, combined age-groups were built with children aged 8-to-9 years in group 1, children aged 10-to-11 years in group 2 and children aged 12 years in group 3. When the 3 age-groups were considered in the analyses, results of SCR again returned a significant Stimulus Type x Age interaction ($F(6,1167) = 2.84$, $p = .019$, $\eta^2 = .01$) with a Stimulus type x Age group quadratic trend ($F(2,389) = 5.67$, $p = .004$, $\eta^2 = .03$). Within-group follow-up tests revealed linear trends in group 1 ($F(1,161) = 3.95$, $p = .048$, $\eta 2 = .02$) and group 2 ($F(1,177) = 6.77$, $p = .010$, $\eta 2 = .04$), but not in group 3.

Interestingly, compared to younger children, 10-to-12-year-olds showed heightened SCRs to GS2 (and somewhat to GS4) resulting in a “zigzag” curve with the highest peak to GS2 (Figure 2.2.2 A vs. Figure 2.2.2 B showing the fear generalization gradient except GS2, GS4).

In sum, results suggest that according to (psycho-physiological) arousal there were differences between children at different ages (8 to 12 years) with more linear trends in

younger children compared to a more quadratic trend in 12-years-old children. This indicates better stimulus discrimination in 12-years-olds as compared to younger children.

A



B

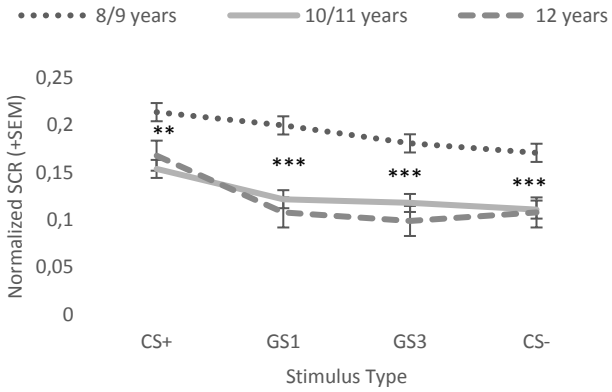


Figure 2.2.2: Fear generalization gradients of SCR of the three age groups (group 1: children aged 8-to-9 years; group 2: children aged 10-to-11 years; group 3: children aged 12 years) with A showing the fear generalization gradient of CS+, CS- and GS1-4. and B showing the fear generalization gradient except GS2, GS4. If GS2 and GS4 were excluded from analyses, more linear curves were seen in younger children indicating more fear generalization in younger children and best discrimination in 12-years-old children; ***p < .001; **p < .01; *p < .05, #p < .10

2.2.2.3 Effects of Awareness

The above analyses included the between-subject factor awareness, but results concerning awareness are reported here for the sake of clarity since no awareness by age effects were found. Contrary to study 1.1, in study 1.2 effects of awareness were found not until generalization. No effects regarding SCR were found.

For valence ratings, fear generalization gradients differed as a function of awareness (Stimulus type x Awareness ($F(5,1925) = 5.13$, $p = .001$, $\eta^2 = .01$) with differences in the linear component between aware and unaware participants (Stimulus type x Awareness linear trend: $F(1,385) = 9.74$, $p = .002$, $\eta^2 = .03$). Within-group follow-up tests revealed linear ($F(1,278) = 9.38$, $p = .002$, $\eta 2 = .03$) and quadratic ($F(1,278) = 4.33$, $p = .038$, $\eta 2 = .02$) trends in aware children and a linear trend ($F(1,106) = 7.21$, $p = .008$, $\eta 2 = .06$) in unaware children. Between-group follow-up tests indicated that children, who were considered to be aware, showed significantly higher valence ratings to CS- and GS4 as compared to children, who were considered to be unaware (Table 2.2.3 and Figure 2.2.3 A).

Table 2.2.3: Valence and Arousal ratings at generalization separated for aware and unaware participants

	VALENCE			**AROUSAL**		
Stimulus	***Aware***	***Unaware***		***Aware***	***Unaware***	
	M (SD)	*M (SD)*	*T- value*	*M (SD)*	*M (SD)*	*T-value*
CS+	4.21 (2.22)	4.66 (2.40)	1.80	5.48 (2.71)	4.53 (2.78)	**-3.12****
GS1	4.78 (2.13)	5.10 (2.25)	1.31	5.02 (2.69)	4.16 (2.59)	**-2.90****
GS2	5.48 (2.02)	5.50 (2.09)	0.08	3.97 (2.48)	3.60 (2.37)	-1.37
GS3	6.05 (1.84)	5.64 (2.12)	-1.91	3.40 (2.35)	3.67 (2.27)	1.03
GS4	6.21 (1.91).	5.74 (2.22)	**-2.10***	3.03 (2.03)	3.55 (2.41)	**2.03***
CS-	6.29 (2.0)	5.83 (2.17)	**-2.01***	3.14 (2.21)	3.91 (2.54)	**2.82****

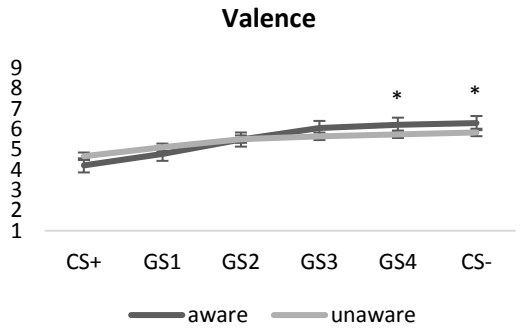
Note: Means (M) and Standard deviations (SD) of valence (1 = very unpleasant, 9 = very pleasant) and arousal (1 = very calm, 9 = very arousing) ratings. Relevant t-values indicate differences between aware and unaware participants. For valence ratings, both phases were considered resulting in mean values between phase 1 and 2. For arousal ratings, however, only values after the second phase were considered here, because differences between aware and unaware participants were mostly due to ratings after the second phase. $^{**}p < .01$; $^{*}p < .05$

Fear generalization gradients for arousal ratings also differed as a function of awareness (Stimulus Type x Awareness interaction: $F(5,1925) = 10.86$, $p < .001$, $\eta 2 = .03$; Stimulus Type x Phase x Awareness interaction: $F(5,1925) = 2.28$, $p = .048$, $\eta 2 = .01$) with differences in the linear component between aware and unaware participants (Stimulus type x Awareness linear

trend: $F(1,385) = 21.42$, $p < .001$, $\eta^2 = .05$; Stimulus type x Phase x Awareness linear trend: $F(1,385) = 4.20$, $p = .041$, $\eta^2 = .01$). Within-group follow-up tests revealed linear ($F(1,278) = 8.17$, $p = .005$, $\eta 2 = .03$) and cubic ($F(1,278) = 6.24$, $p = .013$, $\eta 2 = .02$) trends in aware children, but no effect in unaware children. Between-group follow-up tests indicated that children, who were considered to be aware, showed significantly higher arousal ratings to CS+ and GS1 but lower arousal ratings to CS- and GS4 in phase 2 only, indicating better stimulus discrimination in aware children (Table 2.2.3 and Figure 2.2.3 B).

Taken together, results according to contingency awareness indicate that regarding ratings of valence and arousal aware participants exhibited steeper generalization gradients, but children aged 8 to 12 years did not differ in awareness effects.

A



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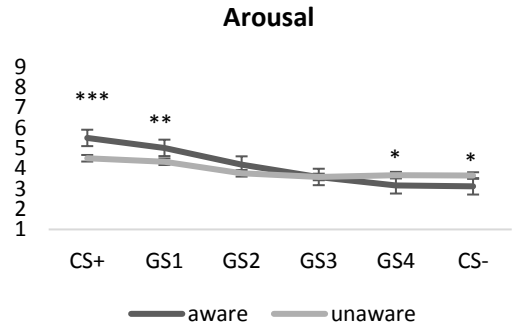


Figure 2.2.3: Differences between aware and unaware participants in fear generalization gradients of (A) valence and(B) arousal ratings; ***p < .001; **p < .01; *p < .05, #p < .10

2.2.2.4 Acquisition and Generalization Effects in Males and Females

Since significantly more females than males were aware of the CS-UCS contingencies after generalization and since sex modulated arousal ratings, it might be important to consider generalization effects separately for males and females. Therefore, we repeated the above analyses separately for males and females. Important age differences at generalization in males and females are reported next.

The observed Stimulus Type x Phase x Age interaction in arousal ratings at generalization resulted from males ($F(20,935) = 1.75$, $p = .022$, $\eta 2 = .04$), indicating that compared to phase 2 boys aged 8 years had higher arousal ratings in phase 1 to GS2 ($t(39) = 2.57$, $p = .014$), boys aged 9 years showed higher arousal to GS4 in phase 2 ($t(36) = -2.07$, $p = .046$), whereas boys aged 11 years showed higher arousal ratings to GS4 in phase 1 ($t(40) = -2.10$, $p = .042$). Boys aged 10 years showed higher arousal ratings to CS+ in phase 1 ($t(60) = 2.05$, $p = .045$). Males aged 12 years showed no significant differences in arousal ratings between phase 1 and phase 2 (Table 2.2.4). No such effects were found for females.

Table 2.2.4: Arousal ratings of boys during generalization separated for phase (1,2) and age (8-12 years)

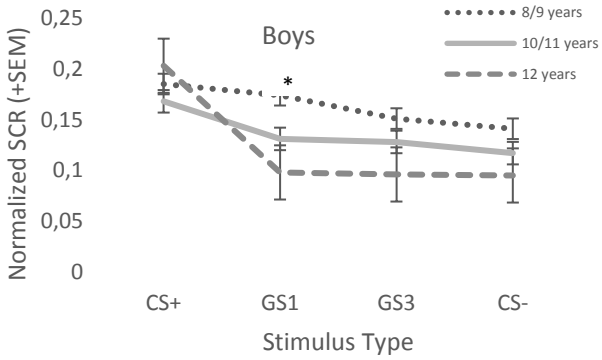
Age	**Stimuli**											
	Phase 1						Phase 2					
	CS+	GS1	GS2	GS3	GS4	CS-	CS+	GS1	GS2	GS3	GS4	CS-
	M (SD)						M (SD)					
8	4.75 (2.87)	4.53 (2.89)	**4.18 (2.67)***	3.50 (2.48)	3.70 (2.64)	2.75 (2.16)	4.73 (2.62)	5.03 (2.91)	3.10 (2.55)	3.35 (2.58)	3.23 (2.44)	3.25 (2.48)
9	4.22 (2.88)	4.68 (2.95)	3.57 (2.70)	3.14 (2.42)	2.73 (2.28)	2.86 (2.19)	4.70 (2.95)	4.14 (2.70)	3.92 (2.59)	3.38 (2.62)	**3.59 (2.68)***	3.62 (2.73)
10	**5.39 (2.65)***	4.74 (2.54)	4.10 (2.53)	3.36 (2.20)	3.25 (2.34)	2.84 (1.96)	4.79 (2.71)	4.56 (2.62)	3.74 (2.36)	3.23 (2.22)	2.82 (1.67)	3.07 (1.98)
11	4.95 (2.92)	4.54 (4.78)	3.93 (2.47)	3.20 (2.16)	**3.51 (2.48)***	3.37 (2.40)	4.88 (2.89)	4.54 (2.65)	3.59 (2.40)	3.51 (2.17)	2.71 (1.98)	3.10 (2.39)
12	3.89 (2.49)	4.78 (2.32)	4.67 (2.52)	4.0 (2.25)	3.83 (2.43)	3.50 (2.07)	4.33 (2.74)	4.06 (1.73)	4.50 (2.04)	3.50 (2.18)	3.06 (2.18)	3.17 (1.89)

Note: Means (M) and Standard deviations (SD) of arousal ratings (1 = very calm, 9 = very arousing) at phase 1 and phase 2 separated per age (8 to 12 years old). * indicate differences between phase 1 and 2 with $p < .05$.

With regard to *SCR*, a Stimulus Type x Age interaction were found for males ($F(20,935) = 2.06$, $p = .013$, $\eta^2 = .04$) and females ($F(20,945) = 3.24$, $p < .001$, $\eta^2 = .06$). In boys, there were differences between the age-groups to **GS1** ($F(4,196) = 3.14$, $p = .016$), and **CS-** ($F(4,196) = 2.92$, $p = .022$), whereas in girls there were differences between the age-groups to **CS+** ($F(4,198) = 6.19$, $p < .001$), **GS1** ($F(4,198) = 7.29$, $p < .001$), **GS3** ($F(4,198) = 8.37$, $p < .001$), **GS4** ($F(4,198) = 3.43$, $p = .010$), and **CS-** ($F(4,198) = 6.97$, $p < .001$), which was similar to differences in the whole sample. Additionally, comparable to the whole sample, a significant main effect of age ($F(1,189) = 3.98$, $p = .004$, $\eta^2 = .08$) indicated that SCR in girls varied between ages with reduced SCR with increasing age. No such main effect of age was found for boys.

Age-differences for SCR regarding fear generalization gradients for girls and boys are depicted in Figure 2.2.4. A, B

A



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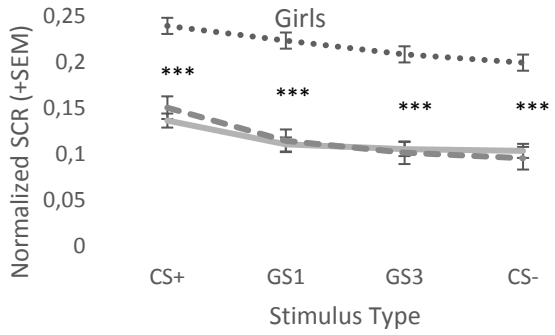


Figure 2.2.4: Fear generalization gradients (except GS2,GS4) of SCR of the three age groups (group 1: children aged 8-to-9 years; group 2: children aged 10-to-11 years; group 3: children aged 12 years) with A showing the fear generalization gradient of boys, and B showing the fear generalization gradient of girls; ***p < .001; **p < .01; *p < .05, #p < .10

In sum, age trends in fear generalization were seen in boys and girls, but best discrimination was found in 12-year-old boys.

2.2.2.5 Regression Analysis in Males and Females

Statistics of the regressions analysis of fear conditioning measures for males and females are shown in Table 2.2.5 and Table 2.2.6, respectively.

Since there were differences between aware and unaware participants as well as between males and females in fear generalization, we further examined whether subjective and physiological arousal at fear conditioning and generalization were associated with age after controlling for awareness, separated for males and females. As described above, we conducted stepwise regressions analyses with variables of the late acquisition and generalization phase, entering awareness after acquisition and generalization, respectively in the first step, subjective arousal in the second step, and SCR in the last step with age as dependent variable.

In males, SCR to CS- after acquisition (as well as after generalization) predicted 24% of the variance (β = -.24; $t(191) = -2.75$, $p = .007$). The overall model during acquisition ($F(5,196) = 3.31$, $p = .007$) was significant, but was not significant during generalization ($p = .163$).

Table 2.2.5: Regression analysis of fear conditioning measures during acquisition in males

	R^2	R^2 change	T value	p-value
Awareness	0.02	0.01	1.83	.068
Arousal to CS+	0.02	0.01	-0.58	.564
Arousal to CS-	0.04	0.02	1.81	.072
SCR to CS+	0.04	0.02	0.61	.546
SCR to CS-	0.08	0.06	-2.75	.007**

** p<.01

In females, SCRs to CS+ ((β = -.22; $t(194) = -2.52$, $p = .013$) as well as CS- (β = -.19; $t(193) = -2.17$, $p = .031$) predicted for age after controlling for awareness. SCR to CS+ predicted 22% (17% at generalization, respectively) of the variance and SCR to CS-predicted 19% (17% at generalization, respectively) of the variance at acquisition. The overall model during

acquisition ($F(5, 198) = 6.08$, $p < .001$) and generalization ($F(5, 198) = 4.34$, $p = .001$), was significant.

Table 2.2.6: Regression analysis of fear conditioning measures during acquisition in females

	R^2	R^2 change	T value	p-value
Awareness	0.00	-0.00	1.14	.255
Arousal to CS+	0.00	-0.00	-0.86	.389
Arousal to CS-	0.00	-0.01	0.29	.774
SCR to CS+	0.12	0.10	-2.52	.013*
SCR to CS-	0.14	0.11	-2.17	.031*

* p<.05

To conclude, the present study demonstrates that there were age differences in fear generalization according to (physiological) arousal. After all dependent measures indicate successful conditioning effects in children at all ages, SCRs indicate differences in fear generalization with best fear discrimination in 12-year-old children with a quadratic gradient, comparable to that of adults. These age differences were seen in boys and girls, but best discrimination was seen in 12-year-old boys. Girls at an early age (under 10 years of age) showed overall higher subjective and physiological arousal compared to older girls (or boys). Results might fit to the fact that prevalence rates for getting anxiety disorders are higher in women compared to men. Additionally, in males, SCR to the safety stimulus was the best predictor of age indicating that safety signal learning depends on age in male subjects. In females, however, SCR to CS+ and CS- predicted age revealing the above mentioned main effect of age in SCR in females.

3. Influences of children's trait anxiety relating to fear learning and its generalization

Based on observations that increased fear generalization has been reported to be associated with AD (e.g. Lissek et al., 2010; Lissek et al., 2014b), and that high trait anxiety is associated with impaired cue differentiation (e.g. Barrett & Armony, 2009; Kadosh et al., 2015) and might predispose for AD (Chambers et al., 2004), we proposed that the developmental trajectory from increased trait anxiety in childhood to an AD could be mediated by abnormal fear conditioning and generalization processes. More specifically, we hypothesized that higher trait anxiety is associated with overgeneralization of conditioned fear.

3.1 Methods

3.1.1 Participants

A sample from the same pool as in study 1.2 was used for this study (473 children aged 8-12 years; 240 females; mean age: 9.7; *SD*: 1.29). All children underwent the described fear conditioning and fear generalization paradigm and had to answer to questionnaires (STAIC trait version and ZLEL, as described in section 1.4). Six children canceled the experiment at acquisition, and additionally 12 children canceled at generalization. Additionally, 49 children were excluded from analysis due to technical errors with physiological recordings. Three children were excluded because of omissions in STAIC questionnaire. Thus, a total of 393 children (199 (50.6%) female; mean age: 9.8; *SD*: 1.3) were included in the study. Descriptive characteristics are given in Table 3.1.

Table 3.1: Descriptive characteristics of the sample

N	STAIC Min	STAIC Max	STAIC Mean (SD)	Negative LE (%)	Awareness Acquisition (%)	Awareness Generalization (%)
393	20	50	29.34 (6.25)	70.5	53	72

Note: Minimum (Min), Maximum (Max), as well as Mean (M) and Standard deviation (SD) of the *Trait scale of the State-Trait Anxiety Inventory for Children (STAIC-T*; Unnewehr, Joormann, Schneider, & Margraf, 1992). The STAIC score was measured on a three-point Likert scale (1) "almost never", (2) "sometimes" and (3) "often", resulting in a sum score between 20 and 60. The German *Zürcher Life-Event List* (*ZLEL*; Steinhausen & Winkler-Metzke, 2001) was used to measure negative life experience (LE).

3.1.2 Statistical Analyses

As already described in chapter 1.4, data were analyzed using repeated-measures ANOVAs with the between-subject factor *STAIC score*, and the within-subject factor *stimulus type* (CS+/CS- at acquisition, CS+/GS-/GS1-4 at generalization), and the additional within-subjects factor *phase*. As before in study 1.1 and study 1.2, contingency *awareness* was integrated as between-subjects factor in the analyses. However, only relevant interaction effects of awareness with trait anxiety were reported here for avoiding replications to study 1.2. Age and sex were set as covariates, since age (p =.022) and sex (p <.001) were correlated with STAIC scores. STAIC scores did not significantly correlate with awareness.

Negative life experience was taken into consideration, since 1) negative life experience (LE) was significantly correlated with STAIC (r =.174, p =.001) and 2) exposure to negative life events, like child maltreatment, was associated with failure to discriminate between threat and safety cues in children and adolescents Thus, to assess the association between subjective and physiological arousal to threat and (ambiguous) safety cues and trait anxiety after controlling for negative life experiences, sex, and age, we conducted stepwise regression analyses with responses to late CS+ and CS- as predictor variables and the trait anxiety score as the dependent variable (cf. Jovanovic et al., 2014). The first regression used fear conditioning variables during acquisition as predictors of trait anxiety score. We entered age, sex, and LE exposure in the first step, arousal to the CS+ in the second step, arousal to CS- in the third step, SCR to CS+ in the fourth step, and SCR to CS- in the final step, doing the same procedure in a second stepwise regression with CS+/CS- during generalization.

3.1 **Results**

3.2.1 (Pre-)Acquisition and Generalization Effects

Results for valence and arousal ratings as well as SCR at pre-acquisition and acquisition are presented in Table 3.2.

3.2.1.1 (Pre-) Acquisition

Across all subjects, the ANOVAs on valence and arousal ratings and SCR revealed no significant stimulus type effects prior to conditioning (ps ≥ .400).

At acquisition, valence and arousal ratings confirmed the expected conditioning effects as the CS+ was rated as more unpleasant than the CS- (significant main effect of Stimulus:

$F(1,338) = 26.44$, $p < .001$, $\eta^2 = .07$; Table 3.2) and as the CS+ relative to the CS- after conditioning elicited enhanced arousal (significant main effect of Stimulus: $F(1,338) = 8.34$, $p = .004$, $\eta^2 = .02$; Table 3.2). Following-up a significant Stimulus Type x STAIC x Awareness interaction ($F(24,338) = 1.60$, $p = .038$, $\eta^2 = .10$) in arousal ratings, post-hoc tests revealed that in unaware participants there were differences according to STAIC for CS+ ($F(25,184) = 1.67$, $p = .032$) and CS- ($F(25,184) = 1.70$, $p = .027$) indicating impaired safety signal learning when trait anxiety scores are high, whereas in aware participants there were significant differences according to STAIC for CS+ ($F(26,207) = 1.62$, $p = .037$) only, indicating higher ratings for CS+ when trait anxiety was high.

For *SCR*, a significant Stimulus Type x STAIC interaction ($F(27,338) = 1.87$, $p = .007$, $\eta^2 = .13$) and a significant Phase x STAIC interaction ($F(27,338) = 2.32$, $p < .001$, $\eta^2 = .16$) were found. However, post-hoc tests yielded no significant effects.

Table 3.2: Valence and Arousal ratings, and SCR of the main sample at pre-acquisition vs. acquisition.

	Stimuli	**Pre-acquisition Ratings M (SD)**	**Acquisition Ratings M (SD)**
Valence	CS+	5.91 (1.98)	4.18 (2.18) ***
	CS-	5.72 (2.09)	6.10 (2.02)
Arousal	CS+	2.98 (2.14)	5.33 (2.47) ***
	CS-	3.02 (2.20)	3.21 (1.95)
SCR	CS+	.18 (.16) *	.20 (.16) ***
	CS-	.16 (.16)	.16 (.14)

Note: Means (M) and Standard deviations (SD) of valence (1 = very unpleasant, 9 = very pleasant) and arousal (1 = very calm, 9 = very arousing) ratings, and SCR; *** indicate differences between CS+ and CS- p<.001; * indicate differences between CS+ and CS- p <.05

3.2.1.2 Generalization

With regard to *valence ratings*, an effect of stimulus did not resist Greenhouse-Geisser correction for multiple testing ($p = .089$), but suggested an upward generalization gradient in valence ratings from CS+ to CS-. A significant main effect of phase ($F(1,338) = 4.80$, $p = .029$, $\eta^2 = .01$) indicated differences between phase 1 and phase 2 with higher ratings of CS- and

GS1-4 in phase 2. A significant Phase x Awareness x STAIC interaction ($F(24,338) = 1.67$, $p = .027$, $\eta^2 = .11$) indicated higher differences between phase 1 and phase 2 in children with low trait anxiety when they were aware. Post-hoc tests, however, reached no significance.

For *arousal ratings*, a significant main effect of stimulus ($p = .04$) did not resist Greenhouse-Geisser correction for multiple testing, but suggested a downward generalization gradient in arousal ratings from CS+ to CS-. Stimulus type x Phase interaction ($F(5,1690) = 2.93$, $p = .014$, $\eta 2 = .01$) with a significant linear trend ($F(1,338) = 9.06$, $p = .003$, $\eta^2 = .03$) only in phase 1 were observed. There were significant differences between arousal ratings in phase 1 and phase 2 to GS2 ($t(392) = 3.42$, $p = .001$; $M_{phase1} = 4.28$, $SD_{phase1} = 2.57$; $M_{phase2} = 3.88$, $SD_{phase2} = 2.45$) and GS4 ($t(392) = 2.17$, $p = .030$; $M_{phase1} = 3.44$, $SD_{phase1} = 2.35$; $M_{phase2} = 3.19$, $SD_{phase2} = 2.15$) with higher ratings in phase 1 indicating a steeper curve in phase 2. A significant main effect of STAIC ($F(27,338) = 1.60$, $p = .032$, $\eta^2 = .11$) revealed that arousal ratings differed according to STAIC with lower ratings in children with low trait anxiety. A significant Stimulus Type x Phase x STAIC interaction ($F(135,1690) = 1.58$, $p < .001$, $\eta^2 = .11$) with differences in the quadratic ($F(24,338) = 1.87$, $p = .006$, $\eta^2 = .13$) an cubic component ($F(24,338) = 1.65$, $p = .025$, $\eta^2 = .12$) indicated that arousal ratings differed according to STAIC for CS+ ($F(27,392) = 1.86$, $p = .007$) and GS1 ($F(27,392) = 1.52$, $p = .049$) in phase 1, and GS1 ($F(27,392) = 1.72$, $p = .016$) and GS4 ($F(27,392) = 1.69$, $p = .019$) in phase 2 with lower arousal ratings when trait anxiety was low.

For *SCR*, there was a significant main effect of stimulus type ($F(5,1690) = 2.69$, $p = .032$, $\eta^2 = .01$) indicating a downward generalization gradient in SCR from CS+ to CS-, but with heightened SCRs to GS2 (and somewhat GS4). Most importantly, the significant interaction of Stimulus type x STAIC ($F(135,1690) = 1.57$, $p < .001$, $\eta 2 = .11$) indicated that generalization gradients differed according to STAIC. High trait anxiety is indicated to be associated with lower SCRs in general, and with highest SCRs to GS2 and GS4. Post-hoc analyses, however, revealed no significant effects.

For better visualizing, we constitute three anxiety groups based on the anxiety trait score of the STAIC score with low anxious (LA) being defined by a score below the 33% percentile, moderate anxious (MA) by scores between the 33% percentile and 66% percentile and high anxious (HA) by scores above the 66% percentile. Differences between these three trait anxiety groups regarding generalization are depicted in Figure 3.1 A–C.

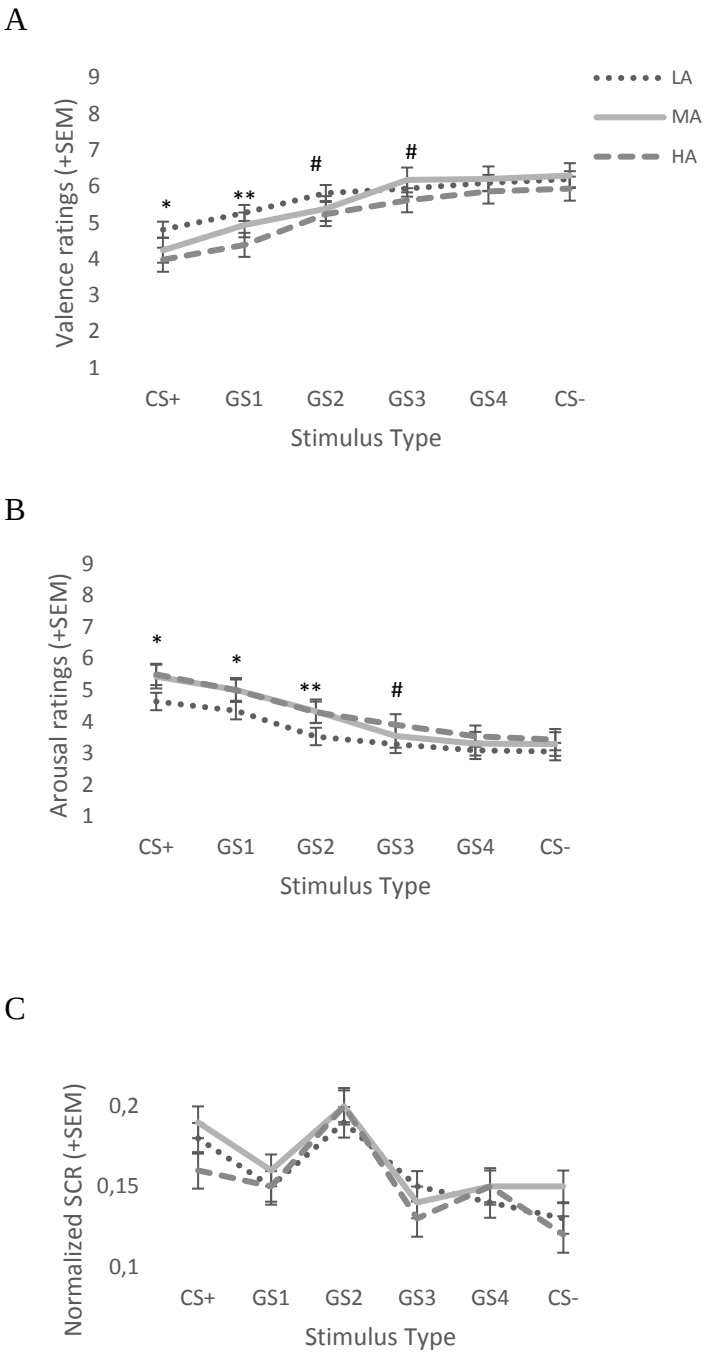


Figure 3.1: Fear generalization gradients of valence (A) and arousal(B) ratings and SCR (C) of the three trait anxiety groups (LA being defined by a score below the 33% percentile, MA defined by scores between the 33% percentile and 66% percentile and HA defined by scores above the 66% percentile); **p < .01; *p < .05, #p < .10

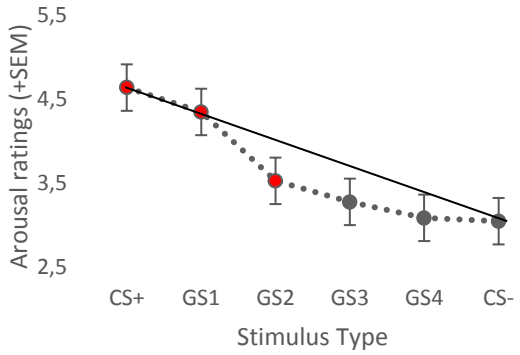
Table 3.3 illustrates group differences in generalization gradients between the three trait-anxiety groups by further obtaining measures of these gradients by relating CS+ and all GSs to CS-. Figure 3.2 visualizes these differences in generalization gradients according to STAIC groups using the example of arousal ratings. HA participants showing a more linear gradient, whereas MA and LA participants showing more quadratic gradients (as indicated by the line).

Table 3.3: Group-specific generalization gradients

	Stimuli	**Differences to CS-** ***t-values***		
		LA	MA	HA
Valence	CS+	-4.97***	-7.12***	-5.86***
	GS1	-3.31**	-5.61***	-5.42***
	GS2	n.s.	-4.49***	-3.36**
	GS3	n.s.	n.s.	n.s.
	GS4	n.s.	n.s.	n.s.
Arousal	CS+	6.73***	9.30***	7.81***
	GS1	6.35***	8.61***	6.32***
	GS2	2.77**	5.79***	3.87***
	GS3	n.s.	n.s.	2.69**
	GS4	n.s.	n.s.	n.s.
SCR	CS+	5.04***	3.23**	3.73***
	GS1	2.90**	n.s.	2.68**
	GS2	4.86***	4.09***	5.77***
	GS3	2.41*	n.s.	n.s.
	GS4	n.s.	n.s.	2.73**

Note: Valence ratings: 1 = very unpleasant, 9 = very pleasant, and Arousal ratings: 1 = very calm, 9 = very arousing; CS- were used as reference; significant results indicate significant differences to CS- in LA (low anxious), MA (moderate anxious) and HA (high anxious) children; * p<.05; ** p<.01; *** p<.001

A



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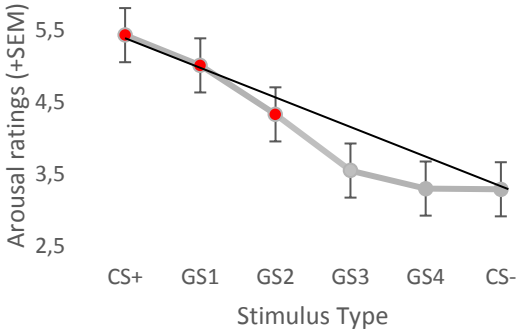


C

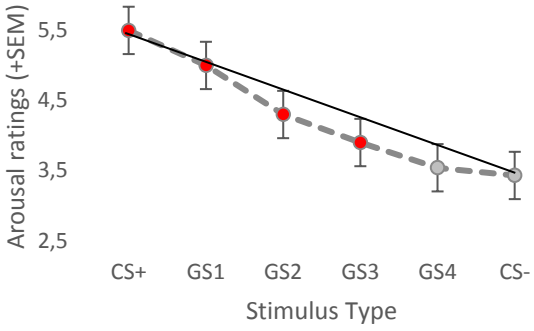


Figure 3.2: Fear generalization gradients of arousal ratings of the three trait anxiety groups ((A) LA being defined by a score below the 33% percentile, (B) MA defined by scores between the 33% percentile and 66% percentile and (C) HA defined by scores above the 66% percentile); red dots indicate significant differences relating to CS-; The lines reflect linear differences between CS- and CS+, demonstrating the deviation of gradients from linearity. **p < .01; *p < .05, #p < .10

3.2.2 Regression Analysis with Negative Life Experience (LE)

Statistics of the regressions analysis of fear conditioning measures at acquisition are shown in Table 3.4.

As described above, we conducted stepwise regressions analyses with variables of the late acquisition and generalization phase, entering age, sex, and LE in the first step, subjective arousal in the second step, and SCR in the last step with trait anxiety score as dependent variable. The overall model during acquisition (F (6, 392) = 10.46, p < .001) and generalization (F (6, 392) = 8.96, p < .001) was significant, accounting for 14% (12% during generalization, respectively) of the variance in STAIC-T score. SCR to CS- at acquisition predicted 16% (t (386) = -2.72, p =.007) of the variance and arousal ratings to CS+ predicted 11% (t (389) =2.14, p =.033) of the variance after controlling for the other variables.

Table 3.4: Regression analysis of fear conditioning measures during acquisition

Predictors for STAIC-T score	**R^2**	**R^2 change**	**F change**	**p-value**
Age, sex, and LE	0.10	0.10	22.23	.001**
Arousal to CS+	0.12	0.11	17.42	.033*
Arousal to CS-	0.12	0.11	13.65	.180
SCR to CS+	0.12	0.11	10.90	.074
SCR to CS-	0.14	0.13	10.46	.007**

Note: LE = negative life experience according to The German *Zürcher Life-Event List* (*ZLEL*; Steinhausen & Winkler-Metzke, 2001). * p<.05; ** p<.01

To sum up, results indicated that 1) arousal were higher in HA children than in LA children, (especial to threat and most ambiguous cues), and 2) contrary to what we expected, children with high trait anxiety did not show significantly more fear generalization than children with lower scores according to valence ratings and SCR. However, there were differences in arousal ratings according to STAIC with fear generalization gradients for HA children were more linear, whereas generalization gradients of LA/MA children were more quadratic. After controlling for age, sex, and negative life experience, arousal ratings to the threat stimulus and SCR to the safety stimulus predicted the trait anxiety level of children.

4. Trait anxiety modulates fear learning and fear generalization in fronto-limbic pathways of the developing brain

Since study 2 indicated that generalization gradients may differ according to trait anxiety, and since alterations in fear conditioning and generalization are considered to play an important role in the pathogenesis of anxiety disorders (e.g. Britton et al., 2013; Lau et al., 2008; Lissek et al., 2010; Lissek et al., 2014b), we wondered whether fronto-limbic morphology during overgeneralization could be defined as biomarkers of early intervention for prevention of anxiety disorders (AD).

In the present study, we analyzed fronto-limbic processes during fear conditioning and generalization in underaged volunteers with different trait anxiety scores. An overactive neuronal fear circuitry and reduced recruitment of prefrontal control have been proposed as neural correlates of facilitated fear conditioning in adults (e.g. Bishop et al., 2004a; Hooker et al., 2008). For example, it has been shown that heightened trait anxiety is related to amygdala dysregulation during processing of aversive and neutral stimuli in healthy subjects (e.g. Bishop, Duncan, & Lawrence, 2004b; Etkin et al., 2004). The relationships between trait anxiety and neurobiological mechanisms of fear learning and generalization in children, however, are largely obscure so far. Additionally, differences in fronto-limbic activation during fear learning and generalization according to sex and developmental aspects are not enough investigated. Since heightened trait anxiety is a risk factor for AD, we hypothesized that high trait anxiety may result in impaired fronto-limbic processing during fear learning and generalization. Furthermore, we hypothesized better stimulus discrimination with increasing age, as reflected by increasing frontal activation with increasing age. We assumed that female subjects showing higher fear generalization reflected by fronto-limbic activation with decreased frontal activation in females compared to males, as consistent with higher comorbidity rates of pathological anxiety in females.

4.1 Methods

4.1.1 Participants

Forty typically developing volunteers, aged 10 to 18 years, participated in the study (17 females; mean age: 12.4; *SD*: 2.0). Additional to the above noted exclusion criteria, valid for all of the experiments in this work, additional exclusion criteria here were neurological diseases, and MRI contraindications (e.g. retainer, braces, claustrophobia).

Participants completed the trait version of the German *State-Trait Anxiety Inventory for Children* (*STAIC*; Unnewehr et al., 1992; see section 1.4). In order to address the influence of trait anxiety on fear conditioning, generalization, and fronto-limbic processes, we defined two anxiety groups based on the median split of the anxiety trait score of the STAIC (Unnewehr et al., 1992). Participants with scores below the median were in the low anxious group (LA), whereas participants with scores above the median were in the high-anxious group (HA). Descriptive statistics auf these two groups are given in Table 4.1.

Table 4.1: Descriptive characteristics of the low and high trait anxiety group

	N Total	**Age M (SD)**	**Female (%)**	**Awareness Acquisition (%)**	**Awareness Generalization (%)**
LA	20	12.0 (1.4)	50	57	86
HA	20	12.8 (2.5)	35	47	73

Note: LA (low anxious), and HA (high anxious) children; LA is defined as participants with scores below the median, whereas HA defined participants with scores above the median. M=Mean; SD= Standard deviation.

STAIC groups did not significantly differ in age (p =. 449), sex (*p* =. 262), and awareness (*p*s ≥.361).

4.1.2 Statistical Analyses

4.1.2.1 Behavioral Data

Data were first analyzed using repeated measures ANOVAs with the between-subjects factor *STAIC group* (LA vs. HA) and the within-subjects factor *stimulus type* (CS+, CS- and additionally GS1, GS2, GS3, GS4 at generalization) separately for each dependent variable (ratings of valence and arousal). Additionally, the within-subjects factor *block* (pre-acquisition (PA), acquisition (A), and generalization (GEN)) was included. Age, sex, and contingency awareness were considered as covariates of no interest.

4.1.2.2 fMRI Data Acquisition, Data Processing and Statistical Analysis

Imaging was performed in a 3 Tesla TIM Trio scanner (Siemens, Erlangen, Germany), equipped with a 12-channel head coil. Whole-brain T2*-weighted Blood-Oxygen-Level-Dependent (BOLD) images were recorded with a gradient echo isotropic echo-planar imaging

(EPI) sequence (repetition time (TR) = 2000 ms, echo time (TE) = 30ms, 420 volumes, 36 slices, 3mm slice thickness, field of view (FoV) = 192mm, flip angle = 90°). Additionally, anatomical images were obtained from each subject, using an isotropic high-resolution T1-weighted 3D structural MRI (sMRI) (Magnetization Prepared Rapid Gradient Echo (MPRAGE) of 176 sagittal slices, and with the sequence parameters TR = 2.3 s, TE = 2.95ms, FoV = 270mm, flip angle = 9°, slice thickness 1.20mm). fMRI data was preprocessed and analyzed with SPM12 (Welcome Trust Centre for Neuroimaging, London, UK). All functional images were slice time corrected, realigned to the first functional volume and unwarped. Images were spatially normalized into a standard stereotactic space (Montreal Neurological Institute, MNI), resampled to isotropic 2 * 2 * 2 mm^3 voxel and spatially smoothed with a Gaussian kernel of 8 mm full width at half maximum (FWHM).

Statistical analyses were based on a general linear model (GLM). The experimental conditions were CS+ and CS- at pre-acquisition (PA), acquisition (A), and generalization (GEN). In addition, a parametric modulation of generalization stimuli GS1 to GS4 ($GEN_{para.}$) was defined to isolate brain activity, which varies in function of similarity to the CS+ (Mean (CS+ and CS-) – Mean (GS1, GS2, GS3 and GS4) cf. Kaczkurkin, 2014). Thus, on a single subject level, onset regressors of the 7 experimental conditions (PA: CS+, CS-, A: CS+, CS-, GEN: CS+, CS-, $GEN_{para.}$) were implemented as regressors of interest within the statistical model as well as realignment parameters as regressors of no interest to correct for motion artifacts. Activation maps were defined for the contrasts PA: CS+>CS-; A: CS+>CS; GEN: CS+>CS-, as well as $GEN_{para.}$: change of activation with similarity to CS+. Resulting contrast-images entered group analyses. In a second step, four 2x2 ANOVA models were defined using *trait anxiety* (HA vs. LA) and *sex* (boys vs. girls) as independent factors, *age* as covariate of interest and brain activation map as dependent variable.

Across all analyses, region-of-interest (ROI)-based analyses were performed focusing on the brain regions as associated with fear learning and generalization (see Introduction), namely PFC regions such as the right and left middle frontal gyrus (MFG), the right and left superior frontal gyrus (SFG), the inferior frontal regions the opercular part (oIFG) and the triangular part (tIFG), the ACC as well as the right and left amygdala and the hippocampus. ROIs were defined using the Wake Forest University PICKATLAS (www.fmri.wfubmc.edu). Group differences were significant when they passed the $p < 0.05$ threshold. To correct for multiple comparisons, the corrected significance level q* as described in the False-Discovery Rate (FDR) procedure by Benjamini and Hochberg (1995) were determined; if $p<q^*$ ($\approx p<.05$ FDR corrected for multiple comparisons) on voxel level, activation was rated as significant.

4.2 **Results**

4.2.1 Behavioral Data

The mean trait anxiety score according to STAIC was 30.55 (*SD* = .611), ranging from 20 to 46. Arousal ratings yielded a significant main effect of block ($F(2,52) = 7.29$, $p = .002$, $\eta^2 = .22$) and a significant Stimulus x Block interaction ($F(2,52) = 4.21$, $p = .020$, $\eta^2 = .14$). Post-hoc tests revealed that participants rated the CS+ significantly higher than CS- after acquisition block ($t(28) = 2.12$, $p = .043$, $M_{CS+} = 3.97$, $SD_{CS+} = 2.06$; $M_{CS-} = 3.05$, $SD_{CS-} = 1.9$) and also after generalization block ($t(27) = 3.75$, $p = .001$, $M_{CS+} = 4.39$, $SD_{CS+} = 2.37$; $M_{CS-} = 2.52$, $SD_{CS-} = 1.67$) but not after pre-acquisition block indicating successful conditioning. Valence ratings yielded a marginal significant main effect of block ($p = .085$). At generalization, a significant main effect of stimulus type ($F(5,130) = 5.86$, $p = .001$, $\eta 2 = .18$) with a significant linear trend ($F(1,22) = 12.86$, $p = .002$, $\eta^2 = .37$) was found for valence ratings indicating a significant upward generalization gradient in valence ratings from CS+ to CS-. Arousal ratings also yielded a significant main effect of stimulus type ($F(5,130) = 6.21$, $p = .001$, $\eta^2 = .19$) with linear ($F(1,26) = 11.09$, $p = .003$, $\eta^2 = .30$) and cubic trends ($F(1,26) = 5.57$, $p = .026$, $\eta^2 = .18$) indicating a downward generalization gradient in arousal ratings from CS+ to CS-.

Table 4.2 demonstrates mean ratings for valence and arousal ratings to CS+ and CS- at (pre-)acquisition and generalization separated per STAIC-group. Significant differences in arousal ratings at acquisition could only be found in the LA group indicating impaired safety-signal learning in HA participants during acquisition. However, both groups could significantly differentiate between CS+ and CS- at generalization. With respect to valence ratings, only the LA group showed a marginally significant effect at acquisition. Neither the LA nor the HA group showed significantly different valence ratings to CS+ /CS- at generalization indicating that according to valence ratings, both groups could not significantly distinguish between the threat and safety stimuli.

Table 4.2: Mean ratings of valence and arousal for CS+ and CS- at pre-acquisition vs. acquisition vs. generalization for the two STAIC groups.

		Valence			**Arousal**		
Block	STAIC-Group	CS+ M (SD)	CS- M (SD)	Test-statistics (two-tailed)	CS+ M (SD)	CS- M (SD)	Test-statistics (two-tailed)
Pre-acquisition	1	6.14 (2.32)	5.86 (1.99)	t=.43	2.71 (2.43)	2.79 (2.29)	t=.19
	2	5.57 (2.65)	5.86 (2.41)	t=.85	2.71 (1.50)	3.29 (2.23)	t=.91
Acquisition	1	4.89 (2.54)	6.36 (2.47)	t=2.04+	4.04 (2.37)	2.29 (1.44)	t=2.81*
	2	5.71 (1.76)	5.00 (2.33)	t=1.36	3.04 (1.79)	3.89 (2.05)	t=.25
Generalization	1	5.39 (2.39)	6.57 (2.34)	t=1.62	4.04 (2.44)	2.36 (1.45)	t=2.35*
	2	4.79 (2.24)	6.11 (2.31)	t=1.66	4.75 (2.34)	2.68 (1.91)	t=2.86*

Note: Mean (M) and Standard deviation (SD) of Valence (1 = very unpleasant, 9 = very pleasant) and Arousal (1 = very calm, 9 = very arousing) ratings.

* indicate differences between CS+ and CS- $p<.05$; + indicate differences between CS+ and CS- $p<.10$

4.2.2 fMRI Data

4.2.2.1 Fear Conditioning and Generalization

Prior to conditioning, the BOLD signal in ROIs did not differ significantly between CS+ and CS-. At acquisition, the CS+ > CS- contrast was significant for the left SFG (x = -42, y = -42, z =10, t = 9.01, p< .001 FWE-corrected). A significant increase in BOLD signal was found in the right SFG (x = 24, y = 46, z = 30, t = 5.38, p < .001 uncorrected) and left (x = 0, y = 18, z = 46, t = 4.10, p <. 001 uncorrected) and right (x = 10, y = 38, z = 26, t = 4.86, p < .001 uncorrected) MFG during generalization with a linear increase of activation to stimuli resembling the CS+ (weakest to CS- with upgraded activity with similarity to CS+).

Table 4.3 demonstrates the ROIs that were significantly differentially activated to CS+ vs. CS- during acquisition and generalization.

Table 4.3. Brain areas responding differentially to CS+ vs. CS- during acquisition and generalization

Block	Brain Region	Side	Contrast	Talairach-Coordinates			t-value
				x	y	z	
Acquisition	SFG	left	CS+>CS-	-42	-42	10	9.01***
Generalization	SFG	right	CS+>CS-	24	46	30	5.38***
	MFG	right	CS+>CS-	10	38	26	4.86***
	MFG	left	CS+>CS-	0	18	46	4.10***

Note: SFG=Superior Frontal Gyrus; MFG= Medial Frontal Gyrus; *** p<.001

4.2.2.2 Influence of Trait Anxiety

There were no significant differences within the ROIs between HA and LA prior to conditioning. However, activation in frontal regions varied as a function of trait anxiety during acquisition and generalization for the CS+ > CS- contrast. At acquisition, HA participants showed reduced activation in the left (x = -42, y = 14, z = 54, t = 2.82, p = .004 uncorrected) and right MFG (x = 46, y = 12, z = 52, t = 4.14, p < .001 uncorrected), and in the ACC (x = 4, y = 42, z = 12, t = 2.52, p = .008 uncorrected).

Generalization processing was reflected by increased activation of HA participants in the left MFG (x = -36, y = 50, z = 14, t = 4.26, p < .001 uncorrected) and right MFG (x = -48, y = 30, z = 38, t = 3.33, p = .002 uncorrected) as well as in the ACC (x = 4, y = 34, z = 0, t = 3.90, p = .001 uncorrected). Parametric generalization analyses showed stronger linear increase in activation to stimuli resembling the CS+ in the left MFG (x = -34, y = 14, z = 42, t = 4.09, p < .001 uncorrected) and the ACC (x = 2, y = 8, z = 26, t = 3.34, p = .002 uncorrected) (Figure 4.1 a, b) in HA participants when compared to LA participants.

Results indicate that HA adolescents showed reduced frontal activation during acquisition, but increased frontal activation during generalization.

a) b)

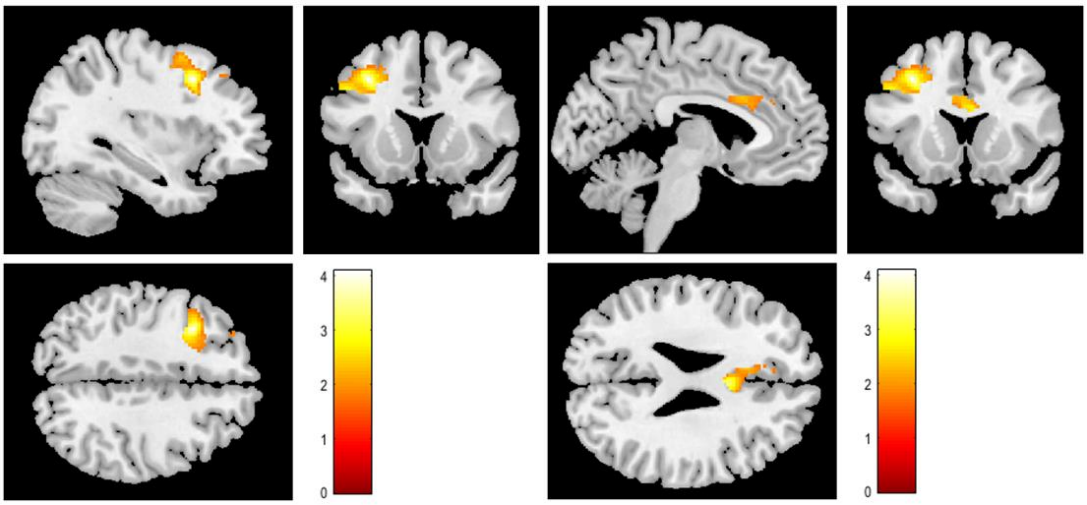
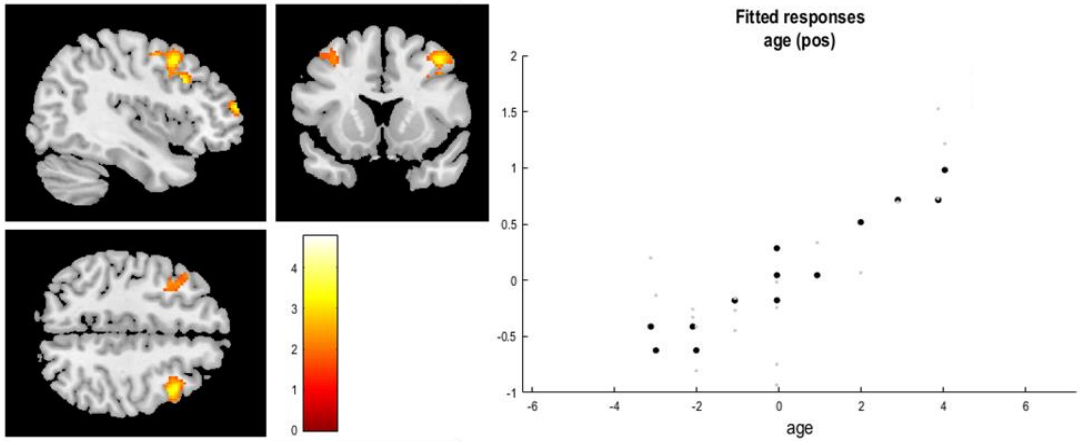


Figure 4.1: Statistical maps of significant relationships between trait anxiety and (a) left MFG (x=-34, y=14, z=42, t=4.09, p<.001 uncorrected) and (b) ACC (x=2, y=8, z=26, t=3.34, p=.002 uncorrected) during generalization to stimuli resembling the CS+. Images threshold at p<.05 uncorrected.

4.2.2.3 Developmental Effects

There were no maturational effects in the processing of CS+ > CS- prior to conditioning. Results indicate that older children showed increased frontal activation during both fear learning and generalization: the older the subjects the stronger the activation with resemblance to CS+ during generalization in left SFG (x=-30, y=54, z=26, t=4.81, p<.001 uncorrected), left (x=-44, y=24, z=44, t=4.42, p<.001 uncorrected) and right MFG (x=24, y=24, z=36, t=5.29, p<.001 uncorrected) as well as the hippocampus (x=-30, y=-32, z=-8, t=2.93, p=.005 uncorrected) (Figure 4.2 a, b).

a)



b)

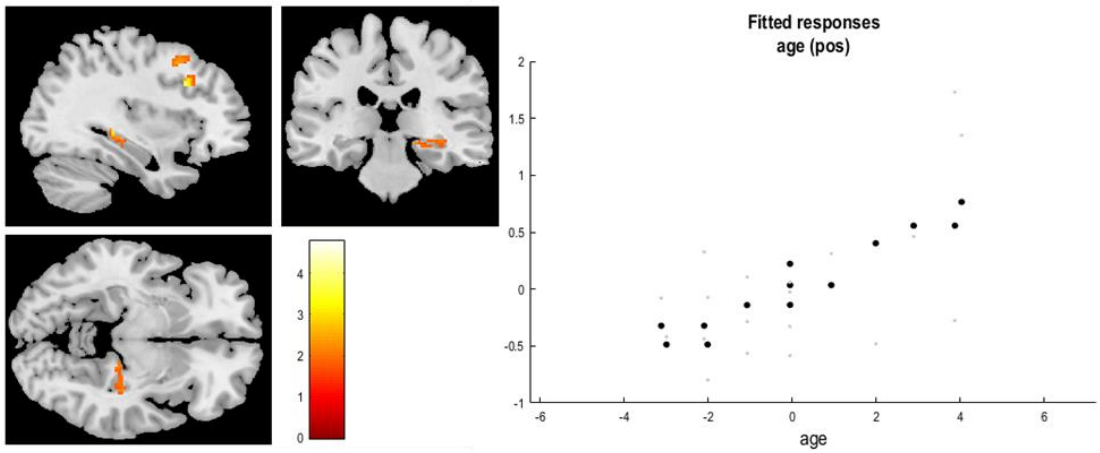


Figure 4.2: Statistical maps of significant relationships between age and (a) left (x=-44, y=24, z=44, t=4.42, p<.001 uncorrected) and right MFG (x=24, y=24, z=36, t=5.29, p<.001 uncorrected), and (b) hippocampus (x=-30, y=-32, z=-8, t=2.93, p=.005 uncorrected) during generalization. Scatter plots showing correlation between individual´s age and (a) MFG and (b) hippocampus activity during generalization. Images threshold at p<.05 uncorrected.

4.2.2.4 Differences according to Sex

At acquisition, there were no significant differences between male and female participants. However, males showed stronger activation the more similar the stimuli were to the CS+ in the hippocampus (x=-18, y=-20, z=-18, t=4.25, p<.001 uncorrected), ACC (x=6, y=30, z=-2, t=3.76, p=.001 uncorrected) and left MFG (x=-22, y=44, z=16, t=3.01, p=.004 uncorrected) during generalization.

To sum up, in the present study, additionally to behavioral data, we used fMRI to identify the neural mechanisms of fear learning and fear generalization investigating also differences in this neural mechanism according to trait anxiety, developmental aspects, and sex. As behavioral data as well as psycho-physiological data already indicated, analyses of fMRI data revealed no significant effects prior to conditioning. After conditioning, analyses revealed 1) significant higher activation in frontal regions to CS+ compared to CS-, and 2) a significant linear increase of activation in these frontal regions to stimuli resembling the CS+ indicating successful fear learning and generalization. Furthermore, significant correlations between trait anxiety and frontal activation were found: At acquisition, HA participants showed reduced activation in left/right MFG and ACC, but at generalization, HA participants showed an increase in these frontal brain regions with stronger linear increase in activation to stimuli resembling the CS+ in HA compared to LA participants. Additionally, significant developmental effects were found: the older the subjects the stronger the hippocampus and frontal activation, with stronger activation to stimuli resembling the CS+. Furthermore, there were differences according to sex: males showed stronger activation to stimuli resembling the CS+ in the hippocampus, left MFG, and ACC when compared to females.

5. General Discussion

From an evolutionary point of view, fear is a highly adaptive mechanism, which allows us to react quickly and appropriately when encountering (potential) threats. Fear conditioning and fear generalization paradigms are excellent models for studying mechanisms underlying fear learning. To simplify, neuroimaging studies in humans during fear conditioning and its generalization showed increased amygdala activation during presentation of stimuli resembling the threat cue, and increased PFC activation during presentation of stimuli with the least resemblance to the safety cue (Dymond et al., 2015; Lissek et al., 2014a). Fear learning and fear generalization appear to be developmental phenomena, which could be attributed to maturational aspects of the developing brain. Contrary to the amygdala, which mature early in life, the vmPFC is one of the last brain regions to mature (Fuster, 2002), and this might be the cause fort the exceeding overgeneralization in younger participants. Overgeneralization of the fear response to ambiguous stimuli may reflect a protective mechanism promoting cautious behavior in childhood, especially in new environments, which decreases with experience during the transitional phase of adolescence, thus, leading to a reduction in generalization with advancing age. However, overgeneralization of conditioned fear in adulthood has been linked to subsequent development of anxiety symptoms (Lenaert et al., 2014) and manifest anxiety disorders (Lissek et al., 2010; Lissek et al., 2014b). Hence, the persistence of overgeneralization of conditioned fear into adulthood could have maladaptive consequences and pose a potential risk mechanism contributing to the emergence of pathological fear.

Within the present thesis, we examined several aspects of fear learning and fear generalization mostly in underage subjects. The first two studies focused on developmental aspects of fear learning and fear generalization: within study 1.1 we investigated differences in fear conditioning and its generalization in healthy children aged 8 to 10 years and healthy adults; in study 1.2 we analyzed developmental differences within children aged 8 to 12 years considering also sex differences. Until today, to my knowledge, no study investigated fear generalization in children, adolescents and adults together in a developmental approach. Thus, with this present thesis we aspired to fill this knowledge gap to some extent by comparing children and adults as well as children and adolescents with the same fear conditioning and generalization paradigm. Furthermore, study 2 examined differences in fear learning and fear generalization according to trait anxiety. In study 3, we further investigated fronto-limbic processes during fear conditioning and fear generalization. Especially, we explored differences

in fronto-limbic processes according to trait anxiety, sex and age during fear learning and generalization.

A superordinate aim of this work was to provide a contribution for a better understanding of the mechanisms of fear learning with the special hope to give potential ideas for preventive approaches of AD and/or individualized treatment. Some novel aspects of the present thesis were that it 1) enabled comparisons of fear generalization gradients between children, adolescents, and adults with the same paradigm, and 2) investigated behavioral, psycho-physiological, and neuronal mechanisms of fear generalization in children according to trait anxiety considering also developmental and sex effects.

In the following section, the key findings of the studies are summarized and discussed in relation to the role of fear learning and fear generalization in the development of AD.

5.1 Key Findings

In the first study, we investigated fear conditioning and its generalization in large samples of healthy children compared to healthy adults. This study constitutes a direct comparison of children and adults regarding acquisition and generalization of conditioned fear using the same paradigm. Most notably, the generalization test convincingly revealed greater fear generalization in children compared to adults as indicated by subjective (verbal ratings) and psycho-physiological (SCR) measures of arousal. Thus, results confirm our hypothesis predicting better cue discrimination with advancing age resulting in heightened fear generalization in children compared to adults. Our far-reaching findings have to be discussed in relation to the results from the acquisition phase indicating that cued fear conditioning in both children and adults leads to similar, robust conditioning effects with higher subjective and psycho-physiological arousal triggered by the CS+ compared to the CS-. We also observed that contingency awareness affected both children and adults in a similar way, with aware participants showing stronger conditioning effects and less generalization, matching our assumptions regarding influences of contingency awareness. Notably, analyses in the subgroup of aware participants only confirmed the main finding that children compared to adults are characterized by enhanced fear generalization. Moreover, we observed several general differences between children and adults, which will be discussed regarding their influences on the observed overgeneralization in children. Especially, we found overall higher physiological arousal in children compared to adults following but not prior to conditioning, and overall more children than adults did not meet criteria for contingency awareness.

The observed difference in fear generalization between children and adults was due to the fact that most GSs triggered more arousal in children compared to adults as reflected in arousal ratings and SCR. As a consequence, children were characterized by a shallower, and to a greater extent linear generalization gradient. We conclude that children are less efficient than adults at recognizing a stimulus (CS+) that during previous learning trials predicted an aversive consequence, and furthermore, at discriminating this stimulus from resembling stimuli (cf. Schiele et al., 2016). The few previous studies on fear generalization in children suggest better discrimination with advancing age (e.g. Glenn et al., 2012a; Michalska et al., 2016). Interestingly, research from animal studies supports our conclusion. Rudy and Pugh (1996), for example, compared auditory-cue fear conditioning in 18-day-old and 25-day-old rats and found more generalized fear in younger than in older rats. While older rats displayed a decline in response strength from the CS+ over the GS to the CS, younger rats were less able to differentiate between the stimuli. The authors interpreted their result with brain maturation effects, suggesting that some brain structures linked with fear conditioning processes are not fully matured in 18-day-old rats. Additionally, Campbell and Haroutunian (1983) used multiple GSs to study perceptual sharpening and showed age-related differences in heart rate orienting response between 16-17 days and 19-20 days of age: older rats were better at discriminating between the stimuli, whereas younger rats showed heightened generalization. Thus, both human and animal research suggest that developmental progress reduces generalization and sharpens discrimination between stimuli.

Importantly, no age group differences were found with regard to the acquisition of conditioned fear. That is, children as reflected in subjective ratings and SCR reliably discriminated between CS+ and CS- and did not differ from adults in this respect. This result corroborates previous results on fear acquisition (e.g. Craske et al., 2008; Glenn et al., 2012a) also showing that children have no deficits in associating a specific cue with an aversive consequence. We conclude that children aged 8 years and older have no deficit in differentiating between stimuli predicting threat and safety as long as there are only two stimuli that can be differentiated easily (cf. Schiele et al., 2016).

The observed overgeneralization of arousal responses to stimuli resembling the CS+ in children seems to be a developmental phenomenon. The maturation of neural structures involved in the neural circuitry of fear learning may play a crucial role. As described in section 1.2.4, neuroimaging studies have highlighted e.g. the role of the amygdala, the hippocampus, and the prefrontal cortex (PFC) in fear learning (LeDoux, 2000; Shin & Liberzon, 2010). These

neural structures are characterized by different trajectories throughout developmental stages (Casey, Jones, & Hare, 2008), with prefrontal regions maturing later than subcortical limbic structures. The phylogenetically ancient subcortical brain regions are shaped by evolution, and from an evolutionary point of view, generalization may increase survival in a generally more dangerous environment encountered by children. Neural structures that evolved at later evolutionary stages and also mature later during ontogenesis might be necessary to inhibit fear responses to ambiguous safety cues (i.e. CS-gradations). As a consequence, children exhibit normal fear conditioning but overgeneralization of conditioned fear with the latter effect becoming ameliorated due to PFC maturation.

A study by Lau et al. (2011) comparing adolescents and adults using fMRI supports this view by revealing that fear learning in adolescents relies to a greater extent on early-maturing subcortical regions (i.e. amygdala, hippocampus) and to a lesser extent on late-maturing prefrontal regions such as the dorsolateral PFC. Thus, PFC involvement in the modulation of fear learning likely increases in the process of brain maturation. Although future research is needed addressing the neurobiological correlates of fear learning and fear generalization longitudinally across the life span from childhood over adolescence to adulthood, we think that data suggests that in children, subcortical regions also respond to stimuli resembling the CS+ resulting in heightened arousal to stimuli that were never associated with threat (cf. Schiele et al., 2016). Thus, lack of PFC maturation may explain the increased physiological arousal in children in response to GSs. Specifically, the ventromedial PFC, one of the last brain regions to mature (Fuster, 2002), is thought to be causally involved in the regulation of SCR (Zhang et al., 2014).

Differences between children and adults in PFC maturation may also explain the observed overall enhanced SCR in children (as also shown in study 1.2 indicating reduced SCR with increasing age in children aged 8 to 12 years). However, this overall group difference cannot account for the observed overgeneralization of arousal responses to GSs in children, but rather seems to be a non-specific response to the experimental paradigm. First, SCR did not differ between adults and children prior to conditioning. Second, no stimulus type by group interaction was found during acquisition, but during generalization. Third, the shallow generalization gradient of children was due to reduced arousal in response to the CS+ but enhanced arousal to most GSs, and this effect cannot be explained by a generally enhanced arousal.

The observed group differences in generalization cannot be explained by contingency awareness either, although more children than adults were considered unaware. The latter finding again may be attributed to PFC maturation in children. Based on findings of Lau et al. (2011), PFC maturation is needed for verbal conditioning effects, which require contingency awareness. Within children and adults, similar effects of awareness on conditioning were found, with stronger conditioning effects and less generalization in aware participants. Since no effect of contingency awareness was observed on group differences regarding generalization, and the same age-group differences in generalization were observed when analyses were performed in aware participants only, we conclude that the lack of PFC maturation rather than lack of awareness in children explain the observed overgeneralization effect (cf. Schiele et al., 2016).

With a second experiment, we built on previous findings of the first study, investigating more precisely developmental trajectories of fear learning and fear generalization by expanding the age range in children. Because a peak of onset of AD can be found during childhood (Kessler et al., 2007), we supposed that there may be a time-window for the development of anxiety disorders as reflected by heightened fear generalization, which is mediated by brain maturation. Thus, this second experiment investigated developmental changes in fear conditioning and fear generalization in children crossing from childhood to adolescents (aged 8 to 12 years). Two key findings will particularly be considered and discussed.

First, according to previous studies in children showing changes in fear learning and generalization with increasing age (e.g. Glenn et al., 2012a; Michalska et al., 2016), we assumed that older children were better at discriminating threat and safety stimuli at fear generalization. Results from the present study based on a large sample support that claim in the tested age range as (psycho-physiological) measures of arousal showed an effect of age at generalization. Interestingly, especially 12-year-old children showed the best stimuli discrimination resulting in a more quadratic gradient when compared to younger children. Thus, hypothesis 2 could be confirmed hypothesizing better stimulus discrimination in participants at early adolescence (12-year-old participants) when compared to children under the age of 12 years. The observed overgeneralization to stimuli resembling the CS+ in children younger than 12 years again supports the notion that this appears to be a developmental phenomenon. As mentioned earlier, cortical brain regions, specifically the vmPFC, which mature later during ontogenesis, might be necessary to inhibit fear responses to ambiguous safety cues (i.e. CS-gradations). Studies concerning on brain development indeed demonstrated that underage participants are characterized by a mature limbic lobe but an under-developed frontal cortex, whereas these

structures are optimally developed in healthy adults resulting in efficient regulation of PFC over the amygdala in adults compared to blunted regulation of fear responses in underage participants (Casey et al., 2010; Gogtay & Thopmson, 2010). Including results of study 1.1, showing greater fear generalization in children aged 8 to 10 years compared to adults, we conclude that children under 12 years might show lack of PFC maturation and/or a shift in connectivity between amygdala and PFC, which may explain the increased physiological arousal in response to GSs. Results of our fMRI study 3 concerning developmental effects of fear learning and generalization support this assumption in that we could show that the older the subjects the stronger they activated frontal regions (especially with increasing similarity of GSs to CS+) during generalization indicating better inhibition of fear reactions to ambiguous safety cues. This could help to explain the fact that most AD usually develop at an early age (Kessler at al., 2007). Since overgeneralization of conditioned fear in adulthood has been linked to subsequent development of anxiety symptoms (Lenaert et al., 2014), heightened fear generalization could pose a potential risk mechanism contributing to the emergence of AD. The age of 12 years seems to play an important role, since generalization gradients at this age were more quadratic similar to gradients in adults.

Interestingly, compared to 8-9-years-olds, participants aged 10-12 years showed heightened SCRs to GS2 (and somewhat to GS4) resulting in a "zigzag" curve with the highest peak to GS2. Whereas children from 10 years on had overall lower SCRs to threat and safety stimuli, the response of these kids to GS2 was as high as in younger children (aged 8 and 9 years) suggesting that children aged 10 years and older were especially anxious in the face of one of the most ambiguous stimuli GS2. As this peak could not be found in adults in study 1.1, this could be a special pattern in adolescents (from 10 years on), which could be linked to the fact that this age range is typically associated with the mean onset of AD (Kessler et al., 2007; Beesdo-Baum et al., 2015). This effect in part could possibly be explained by the phenomena of *novelty effect*. The novelty effect is the tendency for an individual having the strongest stress response when individuals are first faced with a potentially (but ambiguous) threatening experience. This result could imply that children from 10 years on could realize the ambiguity of that stimulus with more resemblance to CS+ than CS-, resulting in higher fear and/or less inhibition of this fear to that stimulus. This could be a result of brain maturation, too, but needs further exploration, e.g. fMRI studies with parallel measure of SCR during fear generalization.

The observed age-group differences in generalization cannot be explained by contingency awareness, because there were no differences in awareness according to age in this

experiment. Thus, contingency awareness affected children at all ages in a similar way, with aware participants showing less generalization confirming again our assumption that participants, who were considered to be aware of the contingency, are better at stimulus discrimination compared to unaware participants. Additionally, similar results concerning fear generalization gradients relating to age emerged if only aware children were considered. Contrary to study 1.1, in study 1.2 we adapted the criterion for being contingency aware, because of high rates of unawareness in children in study 1.1 due to that strict criterion. It has to be considered that, comparable to study 1.1, awareness seems to become important in valence and arousal ratings at generalization, but not in SCR. However, in study 1.1 there were effects of awareness already at acquisition, whereas in study 1.2 awareness was relevant not before generalization. This discrepancy may be a result of an altered criterion for being aware within the children.

A second relevant finding indicated that sex played an important role with differences in arousal ratings already prior to conditioning: Girls showed overall higher arousal ratings (especially to the CS+) than boys. In general, results suggest different developmental aspects of fear learning and generalization relating to sex. Convenient to our assumption that boys are better in discriminating stimuli compared to girls, boys aged 12 years old showed best stimulus discrimination at generalization supporting the notion that prevalence rates for developing AD are higher in females than males. Results of our fMRI study (study 3) also supported this notion in that males showed stronger frontal activation than girls during generalization, especially to stimuli resembling the CS+, indicating stronger fear inhibition to ambiguous (safety) cues.

In rodents, males show greater fear conditioning than females, presumably due to effects of sex hormones emerging at puberty (Dalla & Shors, 2009). Therefore, it might be a deficit of our study not considering effects of sex hormones and/ or menarche or rather phases of girls' menstrual cycle and maturation of brain structures and function. Thus, it is possible that different biological states during testing influenced girls' ratings and/or physiological reactivity. Nevertheless, our results respective sex differences in fear learning are supported by other studies in children and adults. A Study of Gamwell et al. (2015) with children between 8 and 13 years old, for example, also suggested that there are early sex differences in fear conditioning pattern with females showing less discrimination between danger and safety signals compared to age-matched males. Additionally, investigating adults with a fear conditioning paradigm, Lonsdorf et al. (2014) demonstrated robust sex differences in subjective fear ratings with generally higher ratings in women compared to men.

Contrary to females, male participants in our study showed a Stimulus Type x Phase x Age interaction in arousal ratings at generalization indicating that fear generalization according to arousal ratings differed between the age-groups depending on phase in male participants only. Additionally, with regard to SCR at generalization there were significant differences between the age-groups to CS- and GS1 in males, whereas in females, there were differences between age-groups to all stimuli except GS2. But, this result was due to overall higher SCR in younger girls when compared to the middle and older age-groups, whereas in boys, differences according to age were due to higher SCR in response to CS+ but reduced SCR to GSs.

Girls in our study were more often aware of the contingency at generalization indicating that awareness could trigger higher arousal ratings in girls. Interestingly, despite higher awareness in girls, boys were better at discriminating threat vs. safety stimuli. After controlling for awareness, in males, SCR to the safety stimulus was the best predictor of age indicating that safety signal learning depends on age in male subjects, whereas in females, SCR to both CS+ and CS- predicted age revealing a main effect of age in SCR in females with overall higher SCR in younger females. Increased awareness in girls compared to boys might be attributed to varying brain maturation in males and females, since PFC maturation is needed for verbal conditioning effects, which require contingency awareness (Lau et al., 2011).

In a further experiment, we examined the associations between trait anxiety and fear learning and its generalization in children aged 8 to 12 years using SCR and valence and arousal ratings. We hypothesized that higher trait anxiety is associated with overgeneralization of conditioned fear due to impaired (ambiguous) safety signal learning. Our results only in part matching to this assumption and have to be interpreted and discussed in relation to our understanding of the role of trait anxiety in fear learning and its generalization in view of the developmental pathogenesis of AD.

At acquisition, arousal ratings indicated impaired safety signal learning when trait anxiety was high, but only in unaware participants. Results in aware participants indicated higher ratings to the threat cue in HA children, as we would expect. Children with high trait anxiety, indeed, did not show significantly heightened fear generalization according to valence ratings and SCR. However, there were differences in arousal ratings according to STAIC, in which fear generalization gradients for HA children were more linear, whereas generalization gradients of LA/MA children were more quadratic. This goes in line with differences in generalization gradients between healthy adults (showing more quadratic gradients), and

patients with panic disorder (showing more linear gradients) (Lissek et al., 2010). Arousal ratings were overall lower when trait anxiety was low and vice versa. This result is in line with prior studies in underage participants showing that pediatric anxiety was associated with generally higher ratings of fear (Lau et al., 2008). Differences according to STAIC for physiological arousal suggest generally lower SCR when trait anxiety is high, but with a peak to GS2 (and somewhat to GS4). After controlling for age, sex, and negative life experience, arousal ratings to the threat stimulus and SCR to the safety stimulus predicted the trait anxiety level of children.

Our findings to some extent corroborate a previous study, which examined threat perception abnormalities in nonclinical children (Muris, Merckelbach, Schepers, & Meesters, 2003), showing that high levels of trait anxiety went along with enhanced threat perception in response to threat cues. It has to be considered, however, that it could be argued that children in our study with higher trait anxiety scores had also higher arousal ratings due to the fact that both are subjective ratings reflecting the tendency to answer generally higher than other children. However, when controlling for age, sex, and negative life experience, SCR data to CS- predicted children's trait anxiety scores suggesting that dysregulated safety learning may be associated with trait anxiety scores in children, hence, being a risk factor for AD in adulthood. Results in PTSD patients, for example, have found that impaired safety learning may be a specific biomarker of PTSD (Jovanovic & Norrholm, 2011; Jovanovic et al., 2010; Jovanovic et al., 2009). Discrepancies regarding differences in SCRs relative to subjective ratings of arousal are not uncommon and a lot of data reveal that psycho-physiological responses are more unconscious, automatic and reflex-like when compared to conscious ratings. Thus, discrepancies easily arise between automatic psycho-physiological responses and cognitive appraisal of the same stimuli (LeDoux, 2014; Öhman, Carlsson, Lundqvist, & Ingvar, 2007).

Moreover, similar to fear generalization gradients according to SCR in children aged 10-12 years, heightened trait anxiety was associated with highest peaks to GS2 and GS4. This effect again could in part possibly be explained by the *novelty effect*. As already mentioned before, the novelty effect is the tendency for an individual having the strongest stress response when individuals are faced with a potentially threatening experience, especially if this is unpredictable and new. This result is in line with the definition of trait anxiety as reflecting differences in the tendency to evaluate situations as threatening, irrespective of real threat. Of interest is the fact that the other most ambiguous stimulus GS3 showed no heightened SCR, accentuating the role of GS2. This stimulus contains 60% of the CS+ and 40% of the CS-,

making it a most ambiguous cue but closer to CS+ than CS-, possibly resulting in higher fear to that stimulus according to SCR. Both gradients in older children and gradients in HA children showing the highest peak to this most ambiguous cue, matching to the idea that the mentioned age range as well as heightened trait anxiety are both considered to be associated with a risk for developing AD.

Together, high trait anxiety increased fear responses to threat and (ambiguous) safety cues according to subjective ratings of arousal. Furthermore, after controlling for age, sex, and negative life events, SCR to the safety cue (CS-) predicted 16% of the variance of trait anxiety levels suggesting that impaired safety signal learning may be a risk factor for AD in adolescence. Identifying risk phenotypes at an early age may provide opportunities for early prevention of diseases.

For better understanding the neural mechanisms underlying fear learning and generalization, in a further study we used fMRI (additionally to behavioral data) during the differential fear conditioning and fear generalization paradigm. A special aim was to further analyze the neural substrates of fear learning and fear generalization and to investigate these neural mechanisms with respects to trait anxiety, sex as well as developmental effects to achieve a better understanding of the pathogenic mechanisms underlying the development of AD.

As in the above-mentioned studies, behavioral data indicated successful fear conditioning as ratings for CS+ were significantly higher for arousal ratings and marginal significantly lower for valence after conditioning, respectively. Additionally, this CS+ vs. CS- contrast was reflected in the fMRI data. More precisely and in accordance with previous studies in adults (e.g. Schiller et al., 2008; Sehlmeyer et al., 2011), ROI analyses revealed significantly larger activation to CS+ in brain regions associated with learning / inhibition of fear. Furthermore, the fear generalization gradient was represented in the brain data in terms of linear increasing activation in this ROIs to stimuli resembling the CS+.

Additionally, HA participants showed decreased frontal activation for the CS+ vs. CS- contrast during acquisition. Results indicate that HA participants show reduced fear learning or in other words it seems to be harder for participants with high trait anxiety to learn to differentiate between threat and safety stimuli due to less activation of brain areas associated with (inhibitory) cognitive control, thus, resulting in stronger fear. This result is in line with results from our behavioral data of the present study and study 2, indicating impaired safety signal learning in (unaware) participants with high trait anxiety scores. Thus, we argue that high

trait anxiety seems to impair learning processes during the conditioning phase, which may lead to enhanced vulnerability of anxious subjects for developing manifest anxiety or even AD. Contrary to what we assumed, HA participants showed increased activation in frontal regions during generalization, hinting towards a more effortful and cognitively higher loaded processing style in HA compared to LA. This result may be in line with the finding from study 2, demonstrating a tendency for HA children to show generally lower SCRs compared to LA children. Furthermore, contrary to Britton et al., (2013), who demonstrated a u-shaped fear generalization pattern in vmPFC in anxious adolescents, we found linear increased activation in the left MFG and ACC to stimuli resembling the CS+ in healthy adolescents with high trait anxiety. Contrary to results in adults showing reduced frontal activation in anxious participants when appraising threat (Britton et al., 2013; Indovina et al., 2011; Klumpp et al, 2011; Sehlmeyer et al., 2010), adolescents in our study showed even higher frontal activation indicating that there is an hyperregulation in adolescents to compensate the higher difficulties relating differentiation of stimuli resembling the CS+, and this may decompensate with adulthood. We argue that high activation of brain regions, which are associated with inhibitory control over fear related brain areas, could serve as a compensatory mechanism in high trait anxious individuals. This compensatory mechanism could be collapsed in subjects with manifest anxiety disorders and could no longer be found in adults, contributing to the fact that AD usually develop during adolescence. Pediatric anxiety patients showed smaller volumes of amygdala and smaller GM volumes in the PFC when compared to healthy subjects (Milham et al., 2005; Strawn et al., 2013; Wehry et al., 2015). A study with healthy children, on the other hand, showed that high childhood trait anxiety was associated with enlarged volumes (Qin et al., 2014). These findings in healthy HA children are in line with our assumption that this may be interpreted as a morphological correlate of the strong association between threat and ambiguous stimuli, possibly as a compensatory mechanism. In children with anxiety disorders, this compensatory mechanism may collapse consistent with the smaller volumes observed in the clinical populations. Cha et al. (2014), for example, demonstrated that patients with GAD showed a less discriminating vmPFC response during safety vs. threat cues when compared to healthy controls. Results indicated that vmPFC threat processing is closely associated with broader corticolimbic circuit anomalies, which may synergistically contribute to clinical anxiety (Cha et al., 2014).

As expected and already mentioned before, significant developmental effects were found for hippocampus and frontal regions with stronger activation with similarity to the threat cue

with increasing age, especially in male subjects. The former finding shows that the older the subjects the more specifically fronto-hippocampal regions were recruited with resemblance to the threat stimuli (CS+), matching to pervious results showing age-related differences in subcortical and prefrontal regions to threat/safety cue discrimination (Britton et al., 2013; Lau et al., 2011). Maturation of the frontal cortex likely plays a crucial role in the development of AD and other mental disorders (e.g., Mills, Goddings, Clasen, Giedd, & Blakemore, 2014). The PFC is one of the last brain regions to mature (Fuster, 2002), but presumably necessary to inhibit fear responses to ambiguous cues (Kalisch et al., 2006). Numerous studies showed associations between amygdala and PFC function on one side and fear conditioning on the other side, particularly in adults (e.g. Schiller et al. 2008) with only few studies though have been performed in children (Milham et al., 2005; Strawn et al., 2013; Wehry et al., 2015). The latter finding may match with results of our study 1.2 showing higher arousal ratings in girls compared to boys and also with the study of Gamwell et al. (2015) suggesting that there are early sex differences in fear conditioning pattern with females showing less discrimination between danger and safety signals compared to age-matched males. These results in turn may fit to the fact that prevalence rates for developing manifest anxiety disorders are higher for females compared to males.

5.2 Conclusion and Limitations

In sum, many questions still remain open and further studies have to be done. However, this work provides some important findings: 1) Results arguing for a developmental shift of fear generalization with better stimulus discrimination with increasing age. The age of 12 years seems to play an important role in this development, especially because 12 years old subjects were better at discriminating threat vs. ambiguous safety cues than younger children, and showed more quadratic gradients comparable to that of adults. Notably, it has to be considered that there were differences according to sex with 12-years-old boys were best at discriminating stimuli when compared to younger boys and/or girls. Better stimulus discrimination with increasing age was assumed to be due to brain maturation, especially maturation of vmPFC. The vmPFC is one of the last brain regions to mature (Fuster, 2002), and is important for inhibitory control over the amygdala, which is important when considering fear generalization. 2) Beside developmental aspects of fear learning and fear generalization, we found that trait anxiety played a role when considering fear learning and its generalization. Thus, the

mechanisms involved in the development of anxiety disorders are complex and rely on the interplay of many variables, such as environmental, psychological, and neurobiological factors.

Additionally, differently from animals, humans could have explicit cognition about associations. Thus, conscious expecting of threats may involve different processes and modulate the conditioned responses differently compared to participants without such knowledge (Mechias, Etkin, & Kalisch, 2010). In other words, participants, who are aware of the CS-UCS contingency expecting the aversive response, may react differently to those participants, who are considered to be unaware not expecting the aversive response. In our studies, aware participants showed better stimulus discrimination when compared to unaware participants. Hence, awareness might have modulated ratings of valence and arousal. However, the observed differences between children and adults and the age-group differences within children in generalization cannot be explained by contingency awareness either, since contingency awareness affected children at all ages in a similar way, although more children than adults were considered unaware. Furthermore, findings obtained via subjective measures do not necessarily mirror findings obtained via objective ore psycho-physiological measures meaning that even "subjective" unaware participants could show psycho-physiological fear reactions similar to that of "subjective" aware participants.

However, it seems to be useful, to design a novel task and different methods more adapted to children when assessing contingency awareness as well as ratings of valence and arousal for studying fear learning and generalization in children, adolescents and adults with and without AD. First, even though group differences were independent of awareness, the greater percentage of unaware children relative to adults might in part be due to characteristics of the scales used to assess contingency awareness. This may also explain why no effect of awareness on SCR emerged in children in the present or previous studies (Craske et al., 2008), contrary to what has been reported in adults (Lovibond & Shanks, 2002). Second, the present paradigm has been used repeatedly in a similar form in other studies with children, adolescents, and adults (Glenn et al., 2012a; Lau et al., 2008; Lau et al., 2011). However, differences in age-dependent responding to the nature of the stimuli themselves, pictures of female adults, cannot be discarded. Furthermore, consistent with many other studies (e.g. Schiele et al., 2016; Sehlmeyer et al., 2011) we used the subjective measure of valence and arousal ratings as well as contingency ratings only at the end of each experimental block. The continuous measure of ratings, however, would help to investigate direct conditioning-related changes of ratings to stimuli.

It is important to note that this thesis is only concerned with research on human fear learning and generalization, especially research on underage healthy individuals. As a consequence, nonhuman research on fear learning and generalization (e.g. Ciocchi et al., 2010) as well as research on adult individuals only was neglected here. Additionally, it would be interesting to further analyze learning theory models of stimulus generalization and discrimination as well as genetic influencing factors on variability in conditioned fear generalization, however, that was beyond the scope of this thesis.

5.3 Outlook and Clinical Implications

Research on fear learning and fear generalization come more and more to the fore, since overgeneralization was associated with AD, but also with PTSD (Britton et al., 2013; Lau et al., 2008; Lissek & Grillon, 2012; Lissek et al., 2010; Lissek et al., 2014b). In consideration of this and the fact that there is a developmental shift in fear generalization, it is remarkable that there is still only little research on fear generalization in children. Lack of research at this early age-range is remarkable for different reasons. First, as mentioned earlier, the prevalence of AD shows a peak during adolescence (Kessler et al., 2012a). Second, animal studies suggest distinct differences in fear conditioning between adults and adolescents (Baker, Den, Graham, & Richardson, 2014). Thus, the middle childhood (from 8 to 12 years) seems to be a crucial period for studying fear learning because of developmental changes in memory abilities and brain structures, cognition, and social environment (Gee et al., 2013; Ghetti & Bunge, 2012; Gogtay et al., 2004; Ofen, 2012; Riggins, 2014). The lack of research on fear learning and fear generalization in children might be due to ethically reasons and hindered implementation in underage participants. Thus, in fear learning paradigms in children there were high rates of drop outs due to high aversive UCSs (e.g. Britton et al., 2013). An issue complicating fear conditioning research in underage populations pertains to the choice of an effective UCS. In adult samples, a mildly painful electric stimulus is a commonly used UCS that leads to reliable and potent fear responses. However, the use of such an UCS in underage populations is problematic for ethical reasons. Since the strength of the conditioned response depends upon the potency of the UCS, suitable alternatives of comparable strength are required. Recently, a promising alternative has been successfully used in children and adolescents. For instance, Shechner et al. (2015) designed a novel task for examining anxious and healthy children and adults in a more tolerable manner. In this task, a bell was paired with an aversive alarm (UCS) eliciting fear responses, which are tolerable for healthy pediatric and anxious populations.

In any case, more research is needed. For instance, longitudinal follow-ups are required to reveal if and how generalization gradients change through critical developmental time periods and their relationship to the development of anxiety. It would be desirable in the treatment of AD to start at an earlier time point. Thus, chronically manifestations could be inhibited and/or prevention strategies could be created in order to avoid manifest AD.

In addition, future research is needed addressing the neurobiological correlates of fear learning and generalization in a developmental approach. Thus, replication studies are necessary to compare fear learning and generalization, and define fronto-limbic morphology as biomarkers of early intervention or prevention for AD. Replication studies especially in clinical samples are needed to compare fear learning and generalization in children, adolescents, and adults with and without anxiety disorders. As this work analyzed the relations of age, sex and trait anxiety with fear generalization in children including fMRI data, it provides hints that PFC maturation is different according to trait anxiety and sex resulting in different stimuli discrimination. These results could help to generate prevention strategies, as training in stimulus discrimination and/or dealing with negative life experience might help to inhibit fear reactions. Additionally, with a better understanding of the underlying mechanisms of AD, we can provide more individual treatments, e.g. treatments according to sex, and/or age, or define groups at risk for AD, e.g. participants with heightened trait anxiety, to offer them preventative treatments. Along with previous studies showing age-related differences in subcortical and prefrontal regions to threat/safety cue discrimination (e.g. Britton et al., 2013; Lau et al., 2011) we argue that our findings support the suggestion that clinical interventions promoting better stimuli discrimination might be useful during the early period of childhood and adolescence.

Moreover, *extinction* effects should be investigated in further studies since differences were found between healthy and anxious participants with higher fear responses in anxious children compared to non-anxious children (Liberman et al., 2006), and since there were developmental differences in extinction with adolescents showing suppressed extinction compared to both children and adults (Pattwell et al., 2012). In fear extinction paradigms, the CS+ is repeatedly presented without the UCS, thus, eliciting no longer a fear response (Quirk, 2006). Whereas during fear acquisition individuals learn that a stimulus or situation is dangerous, extinction is a mechanism by which individuals learn that something that was previously dangerous has become safe. Furthermore, avoidance behavior of the participants should be mentioned in further studies, since avoidance behavior is often a serious problem in threatening AD prohibiting treatment success. Eye-tracking measure could be a good method for analyzing such

avoidance to threat cues via eye movement measures. It could be very interesting and important to better understand avoidance behavior analyzing the outcomes of attentional biases and attentional avoidance during fear conditioning and generalization.

It may also be important in further studies to involve resilience factors when analyzing risk factors for developing AD. The impact of childhood maltreatment on anxiety symptoms, for example, is thought to interact with genetic variations in a manner that can results in a vulnerable or resilient individual (see e.g. Lesch et al., 1996; Reinelt et al., 2014). Furthermore, self-efficacy (the belief in one´s own ability to successfully cope with adversity; Bandura, 1977) as one advantageous resilience factor has been shown to affect trait anxiety in healthy adolescents (Muris, 2002), and childhood social anxiety (Rudy, Davis, & Matthews, 2012).

As a matter of fact, the present studies constitute a first step in assessing these research questions, since we plan to follow up the examined samples, which can also explain the rather large sample sizes required for the prospective assessment regarding the potential onset of anxiety disorders. One consequence of assessing large sample sizes may be the detection of small magnitude effects often controversially discussed regarding their meaningfulness and replicability. However, there is a clear demand for larger sample sizes and consequently higher statistical power in psychological research in the quest for increasing replicability (Asendorpf et al., 2013).

Finally, it has to be noted that alterations in fear learning and generalization gradients were not only associated with AD, but also with PTSD (Lissek & Grillon, 2012). Additionally, a study of Den et al. (2015) demonstrated that higher levels of depression predicted stronger conditioning in adolescents. Together with the assumption that these alterations were mirrored by altered fronto-limbic processing independent of the specific disorder, it might be suggested that there is evidence for a transdiagnostic relevance for studying fear learning and generalization processes. This matches up with the increasing focus on objective behavioral and neurobiological transdiagnostic measures in mental health research (Morris & Cuthbert, 2012). The National Institute of Mental Health Research Domain Criteria (RDoC) (Sanislow et al., 2010), hence, exemplified a change in the way of classifying psychopathology by cutting across traditional nosologically divisions, rather than clusters signs and symptoms. Dimensional constructs for studying psychopathology according to this RDoC initiative are negative/positive valence systems, cognitive systems, systems for social processes, and arousal/regulatory systems. Consequently, transdiagnostic alterations according to fear learning and generalization might reflect a broader paradigmatic shift in approaches to understanding

mental health problems, hence, providing an excellent context for the translation of novel process-focused interventions from basic research (see Dalgleish & Werner-Seidler, 2014). Therefore, overgeneralization could provide a novel framework for dysregulated emotional circuitry and may led to further intervention (Dunsmoor & Paz, 2015) and prevention strategies.

To close, I would like to cite Frank Thiess (n. d., cited by Beier, 2002, p. 42) "*Angst haben wir alle. Der Unterschied liegt in der Frage wovor*" [free translated: "We are all afraid of anything. The difference lies in the question in front of what we are afraid of."]. This implicates that on the one hand fear is a common, distinct, and highly adaptive emotional state. It activates the defensive fear system in the presence of threat, and thus, it is important for survival of an individual. The motto thereby is "better safe than sorry". On the other hand, however, not only threat cues, but also safety cues have to be identified to avoid permanent arousal. If we consider pathological anxiety and also other psychiatric disorders, e.g. PTSD, overgeneralization from threat cues to similar stimuli, never paired with threat, plays an important role. Thus, investigating the developmental aspects of overgeneralization may lead to a better understanding of the mechanisms of manifest disorders resulting in the provision of prevention strategies. Although there is need for further investigations, the present work gives some first hints for such approaches.

6. References

Asendorpf, J. B., Conner, M., De Fruyt, F., De Houwer, J., Denissen, J. J., Fiedler, K., …, & Wicherts, J. M. (2013). Recommendations for increasing replicability in psychology. *European Journal of Personality, 27*, 108–119.

Bandura A (1977) Self-efficacy: toward a unifying theory of behavioral change. *Psychological Review 84*, 191–215.

Baker, K. D., Den, M. L., Graham, B. M., & Richardson, R. (2014). A window of vulnerability: Impaired fear extinction in adolescence. *Neurobiology of Learning and Memory, 113*, 90-100.

Barrett, J., & Armony, J. L. (2009). Influence of trait anxiety on brain activity during the acquisition and extinction of aversive conditioning. *Psychological Medicine, 39*(2), 255-265.

Beesdo-Baum, K., Knappe, S., Asselmann, E., Zimmermann, P., Brückl, T., Höfler, M., …, & Wittchen, H.-U. (2015). The 'Early developmental stages of psychopathology (EDSP) study': A 20-year review of methods and findings. *Social Psychiatry and Psychiatric Epidemiology, 50* (6), 851-866.

Bishop, S., Duncan, J., Brett, M., & Lawrence, A. D. (2004a). Prefrontal cortical function and anxiety: Controlling attention to threat-related stimuli. *Nature Neuroscience, 7*(2), 184-188.

Bishop, S., Duncan, J., & Lawrence, A. D. (2004b). State anxiety modulation of the amygdala response to unattended threat-related stimuli. *Journal of Neuroscience, 24*, 10364-10368.

Block, J. D., Sersen, E. A., & Wortis, J. (1970). Cardiac classical conditioning and reversal in the mongoloid, encephalopathic, and normal child. *Child Development 41(3)*, 771-785.

Britton, J. C., Grillon, C., Lissek, S., Norcross, M. A., Szuhany, K. L., Chen, G., ..., & Pine, D. S. (2013). Response to learned threat: An fMRI study in adolescent and adult anxiety. *The American Journal of Psychiatry, 170(10)*, 1195-1204.

Britton, J. C., Lissek, S., Grillon, C., Norcross, M. A., & Pine, D. S. (2011). Development of anxiety: The role of threat appraisal and fear learning. *Depression and Anxiety, 28(1)*, 5-17.

Campbell, B. A., & Haroutunian, V. (1983). Perceptual sharpening in the developing rat. *Journal of Comparative Psychology, 97*, 3–11.

Casey, B. J., Jones, R. M., Levita, L., Libby, V., Pattwell, S. S., Ruberry, E. J., ..., & Somerville, L. H. (2010). The storm and stress of adolescence: insights from human imaging and mouse genetics. *Developmental Psychobiology, 52(3*), 225-235.

Casey, B. J., Jones, R. M., & Hare, T. A. (2008). The adolescent brain. *Annals of the New York Academy of Sciences, 1124*, 111–126.

Cha, J., Greenberg, T., Carlson, J. M., DeDora, D. J., Hajcak, G., & Mujica-Parodi, L. (2014). Circuit-wide structural and functional measures predict ventromedial prefrontal cortex fear generalization: Implications for generalized anxiety disorder. *The Journal of Neuroscience, 34*(11), 4043-4053.

Chambers, J. A., Power, K. G., & Durham, R. C. (2004). The relationship between trait vulnerability and anxiety and depressive diagnoses at long-term follow-up of Generalized Anxiety Disorder. *Journal of Anxiety Disorders, 18*(5), 587-607.

Ciocchi, S., Henry, C., Grenier, F., Wolff, S. B. E., Letzkus, J. J., Vlachos, I., …, & Lüthi, A. (2010). Encoding of conditioned fear in central amygdala inhibitory circuits. *Nature, 468*(7321), 277-282.

Cohen, P., Cohen, J., & Brook, J. (1993), An Epidemiological Study of Disorders in Late Childhood and Adolescence—II. Persistence of Disorders. *Journal of Child Psychology and Psychiatry, 34*, 869–877.

Craske, M. G., Waters, A. M., Bergman, R. L., Naliboff, B., Lipp, O. V., Negoro, H., & Ornitz, E. M. (2008). Is aversive learning a marker of risk for anxiety disorders in children? *Behavior Research and Therapy, 46(8)*, 954-967.

Dale, A.M., Fischl, B., & Sereno, M.I. (1999) Cortical surface-based analysis I. Segmentation and surface reconstruction. *Neuroimage 9*, 179-194.

Dalgleish, T., & Werner-Seidler, A. (2014). Disruptions in autobiographical memory processing in depression and the emergence of memory therapeutics. *Trends in cognitive sciences, 18(11)*, 596-604.

Dalla, C., & Shors, T. J. (2009). Sex differences in learning processes of classical and operant conditioning. *Physiology & Behavior, 97(2*), 229–238.

Davis, M. (2000). The role of amygdala in conditioned and unconditioned fear and anxiety. In J. P. Aggleton (Ed.), *The Amygdala*, vol. 2. (pp. 213-287). Oxford: University Press.

Delgado, M. R., Nearing, K. I., LeDoux, J. E., & Phelps, E. A. (2008). Neural circuitry underlying the regulation of conditioned fear and its relation to extinction. *Neuron, 59*(5), 829-838.

Den, M. L., Graham, B. M., Newall, C., & Richardson, R. (2015). Teens that fear screams: A comparison of fear conditioning, extinction, and reinstatement in adolescents and adults. *Developmental psychobiology, 57*(7), 818-832.

Dunsmoor, J. E., & Paz, R. (2015). Fear generalization and anxiety: behavioral and neural mechanisms. *Biological psychiatry, 78*(5), 336-343.

Dunsmoor, J. E., Prince, S. E., Murty, V. P., Kragel, P. A., & LaBar, K. S. (2011). Neurobehavioral mechanisms of human fear generalization. *NeuroImage, 55*(4), 1878-1888.

Dymond, S., Dunsmoor, J. E., Vervliet, B., Roche, B., & Hermans, D. (2015). Fear generalization in humans: Systematic review and implications for anxiety disorder research. *Behavior Therapy, 46*(5), 561-582.

Etkin, A., Klemenhagen, K. C., Dudman, J. T., Rogan, M. T., Hen, R., Kandel, E. R., & Hirsch, J. (2004). Individual differences in trait anxiety predict the response of the basolateral amygdala to unconsciously processed fearful faces. *Neuron, 44*(6), 1043-1055.

Fanselow, M. S. (1994). Neural organization of the defensive behavior system responsible for fear. *Psychonomic Bulletin & Review, 1*(4), 429-438.

Fischl, B., Sereno, M.I., & Dale, A.M. (1999) Cortical surface based analysis II: Inflation, flattening, and a surface-based coordinate system. *Journal of Neuroscience 19*, 8560-8572.

Fullana, M. A., Harrison, B. J., Soriano-Mas, C., Vervliet, B., Cardoner, N., Àvila-Parcet, A., & Radua, J. (2016). Neural signatures of human fear conditioning: An updated and extended meta-analysis of fMRI studies. *Molecular Psychiatry, 21*(4), 500-508.

Fuster, J.M. (2002). Frontal lobe and cognitive development. *Journal of Neurocytology, 31*, 373-385.

Gamwell, K., Nylocks, M., Cross, D., Bradley, B., Norrholm, S. D., & Jovanovic, T. (2015). Fear conditioned responses and PTSD symptoms in children: Sex differences in fear-related symptoms. *Developmental Psychobiology, 57*(7), 799-808.

Gao, Y., Raine, A., Venables, P. H., Dawson, M. E., & Mednick, S. A. (2010). The development of skin conductance fear conditioning in children from ages 3 to 8 years. *Developmental Science, 13(1),* 201-212.

Gazendam, F. J., Kamphuis, J. H., & Kindt, M. (2013). Deficient safety learning characterizes high trait anxious individuals. *Biological Psychology*, *92*(2), 342-352.

Gee, D. G., Humphreys, K. L., Flannery, J., Goff, B., Telzer, E. H., Shapiro, M., …, & Tottenham, N. (2013). A developmental shift from positive to negative connectivity in human amygdala–prefrontal circuitry. *The Journal of Neuroscience, 33(10),* 4584-4593.

Ghetti, S., & Bunge, S. A. (2012). Neural changes underlying the development of episodic memory during middle childhood. *Developmental Cognitive Neuroscience, 2(4),* 381-395.

Ghirlanda, S. & Enquist, M. (2003). A century of generalization. *Animal Behavior, 66,* 15-36.

Glenn, C.R., Klein, D.N., Lissek, S., Britton, J.C., Pine, D.S., & Hajcak, G. (2012a). The development of fear learning and generalization in 8-13-year-olds. *Developmental Psychobiology, 54,* 675-684.

Glenn, C. R., Lieberman, L., & Hajcak, G. (2012b). Comparing electric shock and a fearful screaming face as unconditioned stimuli for fear learning. *International Journal of Psychophysiology, 86(3),* 214-219.

Gloor, P. (1992). Role of the amygdala in temporal lobe epilepsy. In J. P. Aggleton (Ed.), *The amygdala: Neurobiological aspects of emotion, memory, and mental dysfunction* (pp. 505-538). New York: Wiley-Liss.

Gogtay, N., & Thompson, P. M. (2010). Mapping gray matter development: implications for typical development and vulnerability to psychopathology. *Brain and Cognition, 72(1*), 6-15.

Gogtay, N., Giedd, J.N., Lusk, L., Hayashi, K.M., Greenstein, D., Vaituzis, A.C., …, & Thompson, P.M. (2004). Dynamic mapping of human cortical development during childhood through early adulthood. *Proceedings of the National Academy of Sciences of the United States of America, 101,* 8174–8179.

Goldin, P. R., Manber, T., Hakimi, S., Canli, T., & Gross, J. J. (2009). Neural bases of social anxiety disorder: Emotional reactivity and cognitive regulation during social and physical threat. *Archives of General Psychiatry, 66*(2), 170-180.

Greenberg, T., Carlson, J. M., Cha, J., Hajcak, G., & Mujica- Parodi, L. R. (2013a). Ventromedial prefrontal cortex reactivity is altered in generalized anxiety disorder during fear generalization. *Depression and Anxiety, 30*(3), 242-250.

Greenberg, T., Carlson, J. M., Cha, J., Hajcak, G., & Mujica-Parodi, L. R. (2013b). Neural reactivity tracks fear generalization gradients. *Biological Psychology, 92*(1), 2-8.

Haaker, J., Lonsdorf, T. B., Schümann, D., Menz, M., Brassen, S., Bunzeck, N., …, & Kalisch, R. (2015). Deficient inhibitory processing in trait anxiety: Evidence from context-dependent fear learning, extinction recall and renewal. *Biological Psychology, 111*, 65-72.

Haddad, A. M., Bilderbeck, A., James, A. C., & Lau, J. F. (2015). Fear responses to safety cues in anxious adolescents: Preliminary evidence for atypical age-associated trajectories of functional neural circuits. *Journal of Psychiatric Research, 68,* 301-308.

Haddad, A. M., Lissek, S., Pine, D. S., & Lau, J. F. (2011). How do social fears in adolescence develop? Fear conditioning shapes attention orienting to social threat cues. *Cognition and Emotion, 25(6),* 1139-1147.

Hamm, A. O., & Weike, A. I. (2005). The neuropsychology of fear learning and fear regulation. *International Journal of Psychophysiology, 57*, 5-14.

Hofmann S. G., Alpers G. W., & Pauli P., (2008). Phenomenology of panic and phobic disorders. In M. M. Antony, & M. B. Stein, (Eds.), *Handbook of anxiety and related disorders* (pp. 34–46). New York: Oxford University Press.

Hooker, C. I., Verosky, S. C., Miyakawa, A., Knight, R. T., & D'Esposito, M. (2008). The influence of personality on neural mechanisms of observational fear and reward learning. *Neuropsychologia, 46*(11), 2709-2724.

Indovina, I., Robbins, T. W., Núñez-Elizalde, A. O., Dunn, B. D., & Bishop, S. J. (2011). Fear-conditioning mechanisms associated with trait vulnerability to anxiety in humans. *Neuron, 69*(3), 563-571.

Ingram, E., & Fitzgerald, H. E. (1974). Individual differences in infant orienting and autonomic conditioning. *Developmental Psychobiology, 7(4),* 359-367.

Jacobi, F., Höfler, M., Siegert, J., Mack, S., Gerschler, A., Scholl, L., …, & Wittchen, H. (2014). Twelve- month prevalence, comorbidity and correlates of mental disorders in Germany: The Mental Health Module of the German Health Interview and Examination Survey for Adults (DEGS1-MH). *International Journal of Methods in Psychiatric Research, 23*(3), 304-319.

Jovanovic, T., Nylocks, K. M., Gamwell, K. L., Smith, A., Davis, T. A., Norrholm, S. D., & Bradley, B. (2014). Development of fear acquisition and extinction in children: Effects of age and anxiety. *Neurobiology of learning and memory, 113*, 135-142.

Jovanovic, T., & Norrholm, S. D. (2011). Neural mechanisms of impaired fear inhibition in posttraumatic stress disorder. *Frontiers in Behavioral Neuroscience, 5*, 44.

Jovanovic, T., Norrholm, S. D., Blanding, N. Q., Davis, M., Duncan, E., Bradley, B., & Ressler, K. J. (2010). Impaired fear inhibition is a biomarker of PTSD but not depression. *Depression and Anxiety, 27(3*), 244-251.

Jovanovic, T., Norrholm, S. D., Fennell, J. E., Keyes, M., Fiallos, A. M., Myers, K. M., ..., & Duncan, E. J. (2009). Posttraumatic stress disorder may be associated with impaired fear inhibition: relation to symptom severity. *Psychiatry Research, 167(1*), 151-160.

Kaczkurkin, A. (2014). *The Contribution of Fear Conditioning to Pathological Anxiety: An Investigation of Conditioned Fear Generalization in OCD Traits and PTSD*. Doctoral Dissertation, University of Minnesota. Retrieved from https://conservancy.umn.edu/bitstream/handle/11299/167172/Kaczkurkin_umn_0130E_14221.pdf?sequence=1&isAllowed=y

Kadosh, K. C., Haddad, A. M., Heathcote, L. C., Murphy, R. A., Pine, D. S., & Lau, J. F. (2015). High trait anxiety during adolescence interferes with discriminatory context learning. *Neurobiology of Learning and Memory, 12*, 350-357.

Kalisch, R., Korenfeld, E., Stephan, K. E., Weiskopf, N., Seymour, B., & Dolan, R. J. (2006). Context-Dependent Human Extinction Memory is Mediated by a Ventromedial Prefrontal and Hippocampal Network. *The Journal of Neuroscience, 26*(37), 9503-9511.

Kapp, B. S., Whalen, P. J., Supple, W. F., & Pascoe, J. P. (1992). Amygdaloid contributions to conditioned arousal and sensory information processing. In J. P. Aggleton (Ed.), *The amygdala: Neurobiological aspects of emotion, memory, and mental dysfunction* (pp. 229-254). New York, Wiley-Liss.

Kessler, R. C., Avenevoli, S., Costello, E. J., Georgiades, K., Green, J. G., Gruber, M. J., …, & Merikangas, K. R. (2012a). Prevalence, persistence, and sociodemographic correlates of DSM-IV disorders in the national comorbidity survey replication adolescent supplement. *Archives of General Psychiatry, 69(4)*, 372-380.

Kessler, R. C., Petukhova, M., Sampson, N. A., Zaslavsky, A. M., & Wittchen, H. (2012b). Twelve- month and lifetime prevalence and lifetime morbid risk of anxiety and mood disorders in the united states. *International Journal of Methods in Psychiatric Research, 21(3)*, 169-184.

Kessler, R. C., Avenevoli, S., McLaughlin, K. A., Green, J. G., Lakoma, M. D., Petukhova, M., …, & Merikangas, K. R. (2012c). Lifetime co-morbidity of DSM-IV disorders in the US national comorbidity survey replication adolescent supplement (NCS-A). *Psychological Medicine, 42(9)*, 1997-2010.

Kessler, R. C., Amminger, G. P., Aguilar-Gaxiola, S., Alonso, J., Lee, S., & Üstün, T. B. (2007). Age of onset of mental disorders: A review of recent literature. *Current Opinion in Psychiatry, 20*(4), 359-364.

Kim, M. J., Loucks, R. A., Palmer, A. L., Brown, A. C., Solomon, K. M., Marchante, A. N., & Whalen, P. J. (2011). The structural and functional connectivity of the amygdala: from normal emotion to pathological anxiety. *Behavioral Brain Research, 223*(2), 403-410.

Klumpp, H., Ho, S. S., Taylor, S. F., Phan, K. L., Abelson, J. L., & Liberzon, I. (2011). Trait anxiety modulates anterior cingulate activation to threat interference. *Depression and Anxiety, 28*(3), 194-201.

Lau, J. Y., Britton, J. C., Nelson, E. E., Angold, A., Ernst, M., Goldwin, M., …, & Pine, D. S. (2011). Distinct neural signatures of threat learning in adolescents and adults. *PNAS Proceedings of the National Academy of Sciences of The United States of America, 108(11),* 4500-4505.

Lau, J.Y., Lissek, S., Nelson, E.E., Lee, Y., Roberson-Nay, R., Poeth, K., …, & Pine, D.S. (2008). Fear conditioning in adolescents with anxiety disorders: results from a novel experimental paradigm. *Journal of the American Academy of Child and Adolescent Psychiatry, 47*, 94-102.

LeDoux, J. E. (2014). Coming to terms with fear. *Proceedings of the National Academy of Sciences, 111(8*), 2871-2878.

LeDoux, J. (2003). The emotional brain, fear, and the amygdala. *Cellular and Molecular Neurobiology, 23*(4-5), 727-738.

LeDoux, J. E. (2002). *Synaptic self: How our brains become who we are*. New York: Viking.

LeDoux, J. E. (2000). Emotion circuits in the brain. *Annual Review of Neuroscience, 23*, 155–184.

LeDoux, J. E. (1995). Emotion: clues from the brain. *Annual Review of Psychology, 46*, 209–235.

LeDoux, J. E., Farb, C., & Ruggiero, D. A. (1990). Topographic organization of neurons in the acoustic thalamus that project to the amygdala. *Journal of Neuroscience, 10*(4), 1043-1054.

LeDoux, J. E., Farb, C. R., & Romanski, L. M. (1991). Overlapping projections to the amygdala and striatum from auditory processing areas of the thalamus and cortex. *Neuroscience letters, 134(1),* 139-144.

Lenaert, B., Boddez, Y., Griffith, J. W., Vervliet, B., Schruers, K., & Hermans, D. (2014). Aversive learning and generalization predict subclinical levels of anxiety: A six-month longitudinal study. *Journal of Anxiety Disorders, 28*, 747–753.

Lesch K.P., Bengel D., Heils A., Sabol S.Z., Greenberg B.D., Petri S., …, & Murphy D.L. (1996) Association of anxiety-related traits with a polymorphism in the serotonin transporter gene regulatory region. *Science, 274*, 1527–1531.

Liberman, L. C., Lipp, O. V., Spence, S. H., & March, S. (2006). Evidence for retarded extinction of aversive learning in anxious children. *Behavior Research and Therapy, 44(10),* 1491-1502.

Lissek, S., Bradford, D. E., Alvarez, R. P., Burton, P., Espensen-Sturges, T., Reynolds, R. C., & Grillon, C. (2014a). Neural substrates of classically conditioned fear-generalization in humans: A parametric fMRI study. *Social Cognitive and Affective Neuroscience, 9*(8), 1134-1142.

Lissek, S., Kaczkurkin, A.N., Rabin, S., Geraci, M., Pine, D.S., & Grillon, C. (2014b). Generalized anxiety disorder is associated with overgeneralization of classically conditioned fear. *Biological Psychiatry, 75*, 909-915.

Lissek, S. (2012). Toward an account of clinical anxiety predicated on basic, neutrally-mapped mechanisms of pavlovian fear-learning: The case for conditioned overgeneralization. *Depression and Anxiety, 29*(4), 257-263.

Lissek S., & Grillon C., (2012). Learning Models of PTSD. In J. G. Beck, & D. M. Sloan, (Eds.), *The Oxford handbook of traumatic stress disorders* (pp. 175–190). New York: Oxford University Press.

Lissek, S., Rabin, S., Heller, R.E., Lukenbaugh, D., Geraci, M., Pine, D.S., & Grillon, C. (2010). Overgeneralization of conditioned fear as a pathogenic marker of panic disorder. *American Journal of Psychiatry, 167*, 47-55.

Lissek, S., Powers, A. S., McClure, E. B., Phelps, E. A., Woldehawariat, G., Grillon, C., & Pine, D. S. (2005). Classical fear conditioning in the anxiety disorders: a meta-analysis. *Behavior Research and Therapy, 43,* 1391–1424.

Lonsdorf, T. B., Haaker, J., Schümann, D., Sommer, T., Bayer, J., Brassen, S., ..., & Kalisch, R. (2015). Sex differences in conditioned stimulus discrimination during context-dependent fear learning and its retrieval in humans: the role of biological sex, contraceptives and menstrual cycle phases. *Journal of Psychiatry and Neuroscience: JPN, 40*(6), 368-375.

Lovibond, P.F. & Shanks, D.R. (2002). The role of awareness in Pavlovian conditioning: empirical evidence and theoretical implications. *Journal of Experimental Psychology: Animal Behavior Processes, 28,* 3-26.

Maren, S. (2001). Neurobiology of pavlovian fear conditioning. *Annual Review Neuroscience, 24*, 897-931.

Massar, S. A., Mol, N. M., Kenemans, J. L., & Baas, J. P. (2011). Attentional bias in high- and low-anxious individuals: Evidence for threat-induced effects on engagement and disengagement. *Cognition and Emotion*, *25*(5), 805-817.

McDonald, A. J. (1998). Cortical pathways to the mammalian amygdala. *Progress in Neurobiology*, *55*(3), 257-332.

McDonald, A. J., Mascagni, F., & Guo, L. (1996). Projections of the medial and lateral prefrontal cortices to the amygdala: a Phaseolus vulgaris leucoagglutinin study in the rat. *Neuroscience, 71(1*), 55-75.

McLaughlin, K. A., Sheridan, M. A., Gold, A. L., Duys, A., Lambert, H. K., Peverill, M., ..., & Pine, D. S. (2016). Maltreatment exposure, brain structure, and fear conditioning in children and adolescents. *Neuropsychopharmacology, 41*(8), 1956-1964.

Mechias, M. L., Etkin, A., & Kalisch, R. (2010). A meta-analysis of instructed fear studies: implications for conscious appraisal of threat. *Neuroimage*, *49*(2), 1760-1768.

Meloni, E. G., Venkataraman, A., Donahue, R. J., & Carlezon, W. J. (2016). Bi-directional effects of pituitary adenylate cyclase-activating polypeptide (PACAP) on fear-related behavior and c-Fos expression after fear conditioning in rats. *Psychoneuroendocrinology*, *64*, 12-21.

Merz, C. J., Stark, R., Vaitl, D., Tabbert, K., & Wolf, O. T. (2013). Stress hormones are associated with the neuronal correlates of instructed fear conditioning. *Biological Psychology, 92(1)*, 82-89.

Michalska, K. J., Shechner, T., Hong, M., Britton, J. C., Leibenluft, E., Pine, D. S., & Fox, N. A. (2016). A developmental analysis of threat/safety learning and extinction recall during middle childhood. *Journal of Experimental Child Psychology, 146*, 95-105.

Milad, M. R., Quirk, G. J., Pitman, R. K., Orr, S. P., Fischl, B., & Rauch, S. L. (2007). A role for the human dorsal anterior cingulate cortex in fear expression. *Biological Psychiatry, 62*(10), 1191-1194.

Milham, M. P., Nugent, A. C., Drevets, W. C., Dickstein, D. P., Leibenluft, E., Ernst, M., ..., & Pine, D. S. (2005). Selective Reduction in Amygdala Volume in Pediatric Anxiety Disorders: A Voxel-Based Morphometry Investigation. *Biological Psychiatry, 57*(9), 961-966.

Miller, E. K., & Cohen, J. D. (2001). An integrative theory of prefrontal cortex function. *Annual Review of Neuroscience, 24*, 167-202.

Mills, K. L., Goddings, A., Clasen, L. S., Giedd, J. N., & Blakemore, S. (2014). The developmental mismatch in structural brain maturation during adolescence. *Developmental Neuroscience*, *36*(3-4), 147-160.

Monk, C. S. (2008). The development of emotion-related neural circuitry in health and psychopathology. *Development and Psychopathology, 20*(4), 1231-1250.

Morris, S. E., & Cuthbert, B. N. (2012). Research Domain Criteria: cognitive systems, neural circuits, and dimensions of behavior. *Dialogues in clinical neuroscience, 14*(1), 29-37.

Morrow, M. C., Boring, F. W., Keough, T. E., & Haesly, R. R. (1969). Differential GSR conditioning as a function of age. *Developmental Psychology, 1(4*), 299-302.

Mueller, S. C., Aouidad, A., Gorodetsky, E., Goldman, D., Pine, D. S., & Ernst, M. (2013). Gray matter volume in adolescent anxiety: An impact of the brain-derived neurotrophic factor Val^{6} ^{6} Met polymorphism? *Journal of the American Academy of Child and Adolescent Psychiatry*, *52*(2), 184-195.

Muris. P. (2002). Relationships between self-efficacy and symptoms of anxiety disorders and depression in a normal adolescent sample. *Personal Individual Differences 32*, 337–348.

Muris, P., Merckelbach, H., Schepers, S., & Meesters, C. (2003). Anxiety, Threat Perception Abnormalities, and Emotional Reasoning in Nonclinical Dutch Children. *Journal of Clinical Child and Adolescent Psychology*, *32*(3), 453-459.

Neumann, D. L., Waters, A. M., Westbury, H. R., & Henry, J. (2008). The use of an unpleasant sound unconditional stimulus in an aversive conditioning procedure with 8- to 11-year-old children. *Biological Psychology, 79(3),* 337-342.

Ochsner, K. N., Bunge, S. A., Gross, J. J., & Gabrieli, J. D. E. (2002). Rethinking feelings: An fMRI study of the cognitive regulation of emotion. *Journal of Cognitive Neuroscience, 14(*8), 1215-1229.

Ofen, N. (2012). The development of neural correlates for memory formation. *Neuroscience and Biobehavioral Reviews, 36(7)*, 1708-1717.

Öhman, A. (2005). The role of the amygdala in human fear: automatic detection of threat. *Psychoneuroendocrinology, 30*, 953-958.

Öhman, A., Carlsson, K., Lundqvist, D., & Ingvar, M. (2007). On the unconscious subcortical origin of human fear. *Physiology & Behavior, 92(1*), 180-185.

Oldfield, R.C. (1971). The assessment and analysis of handedness: The Edinburgh inventory. *Neuropsychologia 9*, 97-113.

Onat, S., & Büchel, C. (2015). The neuronal basis of fear generalization in humans. Nature *Neuroscience, 18*(12), 1811-1818.

O'Reilly, R. C., & Rudy, J. W. (2001). Conjunctive representations in learning and memory: principles of cortical and hippocampal function. *Psychological Review, 108*(2), 311-345.

Otto, T., & Eichenbaum, H. (1992). Neuronal activity in the hippocampus during delayed non-match to sample performance in rats: Evidence for hippocampal processing in recognition memory. *Hippocampus, 2*(3), 323-334.

Pape, H. C., & Pare, D. (2010). Plastic synaptic networks of the amygdala for the acquisition, expression, and extinction of conditioned fear. *Physiological Reviews, 90*(2), 419-463.

Pattwell, S. S., Duhoux, S., Hartley, C. A., Johnson, D. C., Jing, D., Elliott, M. D., …, & Lee, F. S. (2012). Altered fear learning across development in both mouse and human. *PNAS Proceedings of the National Academy of Sciences of the United States of America, 109(40)*, 16318-16323.

Phan, K. L., Wager, T., Taylor, S. F., & Liberzon, I. (2002). Functional neuroanatomy of emotion: a meta-analysis of emotion activation studies in PET and fMRI. *Neuroimage, 16*(2), 331-348.

Pine, D. S., Cohen, P., Gurley, D., Brook, J., & Ma, Y. (1998). The risk for early-adulthood anxiety and depressive disorders in adolescents with anxiety and depressive disorders. *Archives of General Psychiatry, 55*(1), 56-64.

Pine, D.S. (1997). Childhood anxiety disorders. *Current Opinion in Pediatrics, 9*, 329-338.

Pineles, S.L., Vogt, D.S., & Orr, M.R. (2009). Personality and fear responses during conditioning: beyond extraversion. *Personality and Individual Differences, 46*, 48-53.

Pliszka, S. R., Hatch, J. P., Borcherding, S. H., & Rogeness, G. A. (1993). Classical conditioning in children with attention deficit hyperactivity disorder (ADHD) and anxiety disorders: A test of Quay's model. *Journal of Abnormal Child Psychology, 21(4)*, 411-423.

Qin, S., Young, C. B., Duan, X., Chen, T., Supekar, K., & Menon, V. (2014). Amygdala subregional structure and intrinsic functional connectivity predicts individual differences in anxiety during early childhood. *Biological Psychiatry, 75*(11), 892-900.

Qin, H., & Dubnau, J. (2010). Genetic disruptions of Drosophila Pavlovian learning leave extinction learning intact. *Genes, Brain and Behavior, 9(2),* 203-212.

Quirk, G. J., Garcia, R., & González-Lima, F. (2006). Prefrontal mechanisms in extinction of conditioned fear. *Biological Psychiatry, 60,* 337-343.

Reinelt, E., Aldinger, M., Stopsack, M., Schwahn, C., John, U., Baumeister, S.E., Grabe, H.J., & Barnow, S. (2014) High social support buffers the effects of 5-HTTLPR genotypes within social anxiety disorder. *European Archives of Psychiatry and Clinical Neuroscience, 264,*433–439.

Riggins, T. (2014). Longitudinal investigation of source memory reveals different developmental trajectories for item memory and binding. *Developmental Psychology, 50(2),* 449-459.

Robinson, O. J., Charney, D. R., Overstreet, C., Vytal, K., & Grillon, C. (2012). The adaptive threat bias in anxiety: amygdala–dorsomedial prefrontal cortex coupling and aversive amplification. *Neuroimage*, *60*(1), 523-529.

Rudy B.M., Davis T.E., & Matthews R.A. (2012) The relationship among self-efficacy, negative self-referent cognitions, and social anxiety in children: a multiple mediator model. *Behavior Therapy 43,* 619–628.

Rudy, J. W., & Pugh, C. R. (1996). A comparison of contextual and generalized auditory-cue fear conditioning: evidence for similar memory processes. *Behavioral Neuroscience, 110*, 1299–1308.

Sander, D., Grandjean, D., & Scherer, K. R. (2005). A systems approach to appraisal mechanisms in emotion. *Neural Networks*, *18*(4), 317-352.

Sanislow, C. A., Pine, D. S., Quinn, K. J., Kozak, M. J., Garvey, M. A., Heinssen, R. K., ..., & Cuthbert, B. N. (2010). Developing constructs for psychopathology research: research domain criteria. *Journal of abnormal psychology, 119(4),* 631.

Schafe, G. E., Nader, K., Blair, H. T., & LeDoux, J. E. (2001). Memory consolidation of Pavlovian fear conditioning: a cellular and molecular perspective. *Trends in Neurosciences*, *24*(9), 540-546.

Schiele, M. A., Reinhard, J., Reif, A., Domschke, K., Romanos, M., Deckert, J., & Pauli, P. (2016). Developmental aspects of fear: Comparing the acquisition and generalization of conditioned fear in children and adults. *Developmental Psychobiology*, 58(4), 471-481.

Schiller, D., Levy, I., Niv, Y., LeDoux, J. E., & Phelps, E. A. (2008). From fear to safety and back: Reversal of fear in the human brain. *The Journal of Neuroscience, 28*(45), 11517-11525.

Schiller, J.C.F. (1798). Die Bürgschaft, 1798; Strophe 15, Vers 99; Aus *Wikisource: Die Bürgschaft – Quellen und Volltexte* (Zugriff am 26.08.2017 unter https://de.wikisource.org/wiki/Die_B%C3%BCrgschaft

Schmitz, A., Merikangas, K., Swendsen, H., Cui, L., Heaton, L., & Grillon, C. (2011). Measuring anxious responses to predictable and unpredictable threat in children and adolescents. *Journal of Experimental Child Psychology, 110(2),* 159-170.

Schneider, S., Unnewehr, S., & Margraf, J. (2009). *Kinder-DIPS für DSM-IV-TR. Diagnostisches Interview bei psychischen Störungen im Kindes- und Jugendalter (2. erweiterte und vollständig überarbeitete Auflage)*. Heidelberg: Springer.

Sehlmeyer, C., Dannlowski, U., Schöning, S., Kugel, H., Pyka, M., Pfleiderer, B., ..., & Konrad, C. (2011). Neural correlates of trait anxiety in fear extinction. *Psychological Medicine, 41*(4), 789-798.

Seligman, L. D., Ollendick, T. H., Langley, A. K., & Baldacci, H. B. (2004). The utility of measures of child and adolescent anxiety: A meta-analytic review of the Revised Children's Anxiety Scale, the State-Trait Anxiety Inventory for Children, and the Child Behavior Checklist. *Journal of Clinical Child and Adolescent Psychology, 33(3),* 557-565.

Shechner, T., Britton, J. C., Ronkin, E. G., Jarcho, J. M., Mash, J. A., Michalska, K. J., ..., & Pine, D. S. (2015). Fear conditioning and extinction in anxious and non-anxious youth and adults: Examining a novel developmentally appropriate fear- conditioning task. *Depression and Anxiety, 32(4),* 277-288.

Sheehan, D. V., Lecrubier, Y., Sheehan, K. H., Amorim, P., Janavs, J., Weiller, E., …, & Dunbar, G. C. (1998). The Mini-International Neuropsychiatric Interview (M.I.N.I.): The development and validation of a structured diagnostic psychiatric interview for DSM-IV and ICD-10. *Journal of Clinical Psychiatry, 59* (Suppl. 20), 22–33.

Shin, L. M., & Liberzon, I (2010). The neurocircuitry of fear, stress, and anxiety disorders. *Neuropsychopharmacology, 35*, 169–191.

Somerville, L. H., Whalen, P. J., & Kelley, W. M. (2010). Human bed nucleus of the stria terminalis indexes hypervigilant threat monitoring. *Biological Psychiatry, 68*(5), 416-424.

Spielberger, C. D. (1972). *Anxiety: Current trends in theory and research, vol. I.* Oxford: Academic Press

Spielberger, C.D., Gorsuch, R.L., & Lushene, R.E. (1970). *STAI. Manual for the State-Trait-Anxiety Inventory*. Palo Alto: Consulting Psychologists Press.

Steimer, T. (2002). The biology of fear- and anxiety-related behaviors. *Dialogues in Clinical Neuroscience, 4(3*), 231–249.

Steinhausen, H. C., & Winkler-Metzke, C. (2001). Die Zürcher Lebensereignis-Liste (ZLEL): Ergebnisse einer Schweizer epidemiologischen Untersuchung. *Kindheit und Entwicklung, 10*(1), 47–55.

Strawn, J. R., Hamm, L., Fitzgerald, D. A., Fitzgerald, K. D., Monk, C. S., & Phan, K. L. (2015). Neurostructural abnormalities in pediatric anxiety disorders. *Journal of Anxiety Disorders, 32*81-88.

Strawn, J. R., Wehry, A. M., Chu, W., Adler, C. M., Eliassen, J. C., Cerullo, M. A., …, & DelBello, M. P. (2013). Neuroanatomic abnormalities in adolescents with generalized anxiety disorder: A voxel- based morphometry study. *Depression and Anxiety, 30*(9), 842-848.

Thiess, F. (o.J.). In B. Beier (Eds.), *Harenberg Lexikon der Sprichwörter & Zitate - mit 50000 Einträgen das umfassendste Werk in deutscher Sprache*, (S. 42). Mannheim: Bibliografisches Institut.

Tinoco-González, D., Fullana, M. A., Torrents-Rodas, D., Bonillo, A., Vervliet, B., Blasco, M. J., Farre, M., & Torrubia, R. (2015). Conditioned fear acquisition and generalization in generalized anxiety disorder. *Behavior Therapy, 46*, 627–639.

Torrents-Rodas, D., Fullana, M. A., Bonillo, A., Caseras, X., Andión, O., & Torrubia, R. (2013). No effect of trait anxiety on differential fear conditioning or fear generalization. *Biological Psychology, 92*(2), 185-190.

Tottenham, N., Tanaka, J.W., Leon, A.C., McCarry, T., Nurse, M., Hare, T., …, & Nelson, C. (2009). The NimStim set of facial expressions: Judgments from untrained research participants. *Psychiatry Research, 168*, 242-249.

Unnewehr, S., Joormann, S., Schneider, S., & Margraf, J. (1992). *Deutsche Übersetzung des State-Trait Anxiety Inventory for Children*. Unveröffentlichtes Manuskript.

Walker, D. L., & Davis, M. (2002). The role of amygdala glutamate receptors in fear learning, fear-potentiated startle, and extinction. *Pharmacology Biochemistry and Behavior, 71*(3), 379-392.

Waters, A. M., Henry, J., & Neumann, D. L. (2009). Aversive Pavlovian conditioning in childhood anxiety disorders: Impaired response inhibition and resistance to extinction. *Journal of Abnormal Psychology, 118(2),* 311-321.

Watson, J. B., & Rayner, R. (1920). Conditioned emotional reactions. *Journal of Experimental Psychology, 3(1),* 1-14.

Wehry, A. M., McNamara, R. K., Adler, C. M., Eliassen, J. C., Croarkin, P., Cerullo, M. A., … & Strawn, J. R. (2015). Neurostructural impact of co-occurring anxiety in pediatric patients with major depressive disorder: A voxel-based morphometry study. *Journal of Affective Disorders, 171,* 54-59.

Weiss, R. H. (2006). *Grundintelligenztest Skala 2 - Revision - (CFT 20-R)*. Göttingen: Hogrefe.

Whalen, P. J. (1998). Fear, vigilance, and ambiguity: Initial neuroimaging studies of the human amygdala. *Current Directions in Psychological Science, 7(6*), 177-188.

Zhang, S., Hu, S., Chao, H. H., Ide, J. S., Luo, X., Farr, O. M., & Li, C. S. (2014). Ventromedial prefrontal cortex and the regulation of physiological arousal. Social Cognitive and *Affective Neuroscience, 9*, 900–908.

7. Glossary

Abbreviations	*Definition*
AB	Accessory Basal nuclei of Amygdala
ACC	Anterior Cingulate Cortex
AD	Anxiety Disorders
B	Basal nuclei of Amygdala
BLA	Basolateral nuclei of Amygdala
BOLD	Blood-Oxygen-Level-Dependent
Ce	Central nuclei of Amygdala
CR	Conditioned Response: the response induced by a conditioned stimulus after conditioning
CS	Conditioned Stimulus
CS-	Conditioned Stimulus presented during conditioning, but never associated with the unconditioned stimulus; the so called "safety" stimulus
CS+	Conditioned Stimulus presented during conditioning, and associated with the unconditioned stimulus; the so called "threat" stimulus
DIPS	Diagnostic Inventory of Psychiatric disorders
dACC	dorsal Anterior Cingulate Cortex
dlPFC	dorsolateral Pre-Frontal Cortex
dmPFC	dorsomedial Pre-Frontal Cortex
EPI	Echo-Planar Imaging
FDR	False-Discovery Rate
fMRI	functional Magnetic Resonance Imaging
FoV	Field of View
FWHM	Full Width at Half Maximum
GLM	General Linear Model
GM	Grey Matter
GS	Generalized Stimulus
GAD	Generalized Anxiety Disorder
HA	High-Anxious participants: Participants with scores above the 66% percentile of the anxiety trait score of the STAIC

ICV	Intercranial Brain Volume
ITI	Inter-Trial Interval: the time window between one stimulus´s offset and the next stimulus´s onset
La (Amygdala)	Lateral nuclei of Amygdala
LA	Low-Anxious participants: Participants with scores below the 33% percentile of the anxiety trait score of the STAIC
LE	negative Life Events
MA	Moderate-Anxious participants: Participants with scores between the 33% and 66% percentile of the anxiety trait score of the STAIC
MFG	Middle Frontal Gyrus
MPRAGE	Magnetization Prepared Rapid Gradient Echo
oIFG	opercular part of the Inferior Frontal Gyrus
PFC	Pre-Frontal Cortex
PTSD	Post-Traumatic Stress Disorder
RDoC	Research Domain Criteria
ROI	Region of Interest
SCR	Skin Conductance Response
SFG	Superior Frontal Gyrus
sMRI	structural Magnetic Resonance Imaging
SMA	Supplementary Motor Area
STAI-C	State-Trait-Anxiety Inventory for Children
TE	Echo Time
tIFG	triangular part of the Inferior Frontal Gyrus
TR	Repetition Time
UCS	Un-Conditioned Stimulus: stimulus, which induces unconditioned responses without learning (biologically salient; e.g. a loud scream or an electrical shock)
vlPFC	ventrolateral Pre-Frontal Cortex
vmPFC	ventromedial Pre-Frontal Cortex
ZLEL	Zürcher Life Event List

Terminology	*Definition*
Contingency Awareness	An individual's knowledge of the association between CS+ and UCS: participants can verbalize the reinforcement contingencies within the paradigm
Fear conditioning	The process whereby a neutral stimulus becomes a fearful stimulus (CS), which could elicit a fear response (CR) by repeatedly combined matching with an aversive unconditioned stimulus (UCS) e.g. an electrical shock or a loud tone.
Discriminative fear conditioning	Individuals learn that a conditioned stimulus (CS+) predicts the UCS, while another stimulus (CS-) is never followed by the UCS and predicts safety. The differential fear conditioning paradigm is necessary to validate specificity of learning, and rule out non-associative effects.
Fear generalization	Fear generalization describes a learning process whereby the conditioned fear responses extend to stimuli (generalization stimuli, GSs), which are similar to the CS+, but never followed by the UCS. Heightened (maladaptive) fear generalization in this context is also termed **overgeneralization**

8. Appendix

8.1. Flyer for Advertising of the Study

Einverständniserklärung

☑ Ich bin damit einverstanden, dass die **Kinder- und Jugendpsychiatrie des Universitätsklinikums Würzburg** mit mir Kontakt aufnimmt und mich über die aktuelle Studie informiert.
Das Einverständnis kann ich jederzeit wieder zurückziehen.

Name des Kindes

Geburtsdatum

Name der/des Sorgeberechtigten

Telefonnummer

E-Mail

Datum Unterschrift

Weitere Informationen

Direktion
Prof. Dr. Marcel Romanos

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Anschrift
Klinik und Poliklinik für Kinder- und Jugendpsychiatrie, Psychosomatik und Psychotherapie
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Besuchen Sie unsere Homepage:
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Universitätsklinikum Würzburg

Warum müssen Kinder Angst haben?

Bitte helfen Sie uns das herauszufinden!

50 EURO Aufwands-entschädigung

Kinder zwischen 8 und 12 Jahren für wissenschaftliche Studie gesucht

KJPPP
Klinik und Poliklinik für Kinder- und Jugendpsychiatrie, Psychosomatik und Psychotherapie

Unser Anliegen

Liebe Eltern,

wir wenden uns an Sie, weil wir Sie um die Hilfe Ihrer Familie bitten möchten.

Angsterkrankungen gehören zu den häufigsten psychischen Störungen bei Kindern und führen oft zu Depression im Erwachsenenalter. Um zu verstehen, wie Angsterkrankungen entstehen und wie wir sie verhindern können, untersuchen wir auch gesunde Kinder in einer großen Studie, die von der Deutschen Forschungsgemeinschaft gefördert wird.

Wir wären Ihnen sehr dankbar, wenn Sie uns dabei helfen könnten.

Wer kann teilnehmen?

Kinder zwischen 8 und 12 Jahren.

Was haben Sie und Ihr Kind davon?

Jede Familie erhält für die Teilnahme pauschal 50 Euro.

50 EURO Aufwands-entschädigung

Was wird bei der Studie gemacht?

Noch ist unbekannt, warum Kinder Ängste entwickeln. Daher erfassen wir sehr genau durch eine Befragung und eine psychologische Untersuchung, ob bei Ihrem Kind bestimmte Ängste vorliegen. In einer Untersuchung am Computer wollen wir herausfinden, wie Ängste ausgelöst werden können. Dies ist völlig ungefährlich und die Ethikkommission der Universität Würzburg hat bestätigt, dass die Studie völlig unbedenklich ist. Weil die Erbfaktoren hierbei eine wichtige Rolle spielen, möchten wir auch diese durch eine Blutentnahme (oder auch eine Speichelprobe) untersuchen.
Die gesamte Dauer für die Teilnahme beträgt ca. 3–4 Stunden.

Warum sollen wir teilnehmen?

Bislang existiert weltweit keine vergleichbar große und aufwändige Studie, die darauf abzielt, die Entstehung von Angsterkrankungen zu entschlüsseln. Durch Ihre Bereitschaft, an dieser Studie teilzunehmen, leistet Ihre Familie einen sehr wichtigen Beitrag, um Angsterkrankungen zu verhindern und betroffenen Kindern zu helfen.

Herzlichen Dank für Ihr Interesse und Ihre Hilfe!

Möchten Sie mehr Informationen zur Studie haben?

Gerne nehmen wir unverbindlich mit Ihnen Kontakt auf! Bitte füllen Sie dazu kurz das nachfolgende Formular aus, geben es unterschrieben bei den Lehrern oder Lehrerinnen ab oder schicken Sie es uns zu.

Kontakt
Klinik und Poliklinik für Kinder- und Jugendpsychiatrie, Psychosomatik und Psychotherapie
Fr. Julia Oechsner
Telefon: 0931 / 201-78030
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8.2. Informed Consent Form and Sign-up Sheet of Study 1 und Study 2

8.2.1. Informed Consent Form and Sign-up Sheet for Children

AUFKLÄRUNG UND EINVERSTÄNDNISERKLÄRUNG ZUR TEILNAHME AN DER UNTERSUCHUNG

GENETISCHE EINFLUSSFAKTOREN AUF DIE FURCHTKONDITIONIERUNG UND –GENERALISIERUNG BEI ANGSTSTÖRUNGEN

(FÜR KINDER)

WARUM WIRD DIESE STUDIE GEMACHT?

Viele Kinder, die zu uns ins Krankenhaus kommen, haben Angst vor bestimmten Situationen und sind dadurch sehr belastet. Manche trauen sich nicht vor anderen zu sprechen oder haben große Angst vor Hunden; wieder andere haben so viel Angst, dass sie nicht mehr in die Schule gehen wollen. Wir sind Wissenschaftler an der Universitätsklinik und wir wissen bereits, dass Ängste zum Teil angeboren sind, aber es ist auch wichtig, was die Kinder vorher erlebt haben. Nun möchten wir herausfinden, wie diese Ängste entstehen und wollen dadurch neue Ideen sammeln, wie wir diese Ängste besser behandeln können.
Dabei kannst Du uns helfen!

WAS WIRD IN DIESER STUDIE GEMACHT?

Wir stellen zunächst Dir und Deinen Eltern einige Fragen, um herauszufinden ob bei Dir eine unentdeckte Angsterkrankung besteht. Wir wollen dann auch von Dir wissen, wie es Dir geht und wovor Du Angst hast.
Danach machen wir einen Test, mit dem wir herausfinden wollen, wie Angsterkrankungen entstehen. Der Test ist aber ungefährlich und Du wirst dadurch nicht ängstlicher werden als vorher. Während der eigentlichen Untersuchung zeigen wir Dir auf einem Computerbildschirm Fotografien mit Gesichtern. Manchmal zeigen wir dir die Gesichter mit einem ängstlichen Gesichtsausdruck. Manchmal hörst Du dann auch über einen Kopfhörer ein unangenehmes lautes Geräusch. Das ist zwar kurz unangenehm, aber gar nicht gefährlich. Während der Untersuchung messen wir mit kleinen Knöpfen, wie Dein Körper während dem Test reagiert. Alle Fragebögen und die Untersuchung dauern zusammen nicht mehr als zwei Stunden.
Um zu untersuchen, welchen Einfluss die Vererbung auf die Entstehung von Ängsten hat, möchten wir Dir eine kleine Menge Blut abnehmen (2 Röhrchen). Die Blutentnahme ist ganz ungefährlich und wird nur von Personen gemacht, die das gelernt haben. Wir verwenden Dein Blut nur für diese Studie.

WAS HABE ICH DAVON, BEI DER STUDIE MITZUMACHEN?

Du selbst wirst nichts davon haben, wenn Du an der Studie teilnimmst. Du hilfst uns aber dabei, besser nachzuvollziehen, wie Ängste entstehen und dadurch kann dann in Zukunft möglicherweise Menschen mit Angsterkrankungen besser geholfen werden.

IST DIE TEILNAHME AN DER STUDIE GEFÄHRLICH?

Beim Blutabnehmen kann es an der Einstichstelle zu einem blauen Fleck kommen oder sich die Einstichstelle entzünden. Man kann auch ein falsches Blutgefäß treffen. Diese Probleme passieren aber, wenn man vorschriftsmäßig Blut abnimmt, extrem selten.

WAS PASSIERT MIT DEN DATEN?

Alle Informationen, die wir von Dir und Deiner Familie bekommen, werden streng vertraulich behandelt. Wir stellen sicher, dass keine Unbefugten an diese Informationen gelangen können. Bevor wir die Daten auswerten, oder auch mit anderen Wissenschaftlern gemeinsam auswerten, werden die Informationen, die Blutprobe und die Ergebnisse aus den Tests mit einer speziellen Zahl beschriftet und ohne Deinen Namen weiterverwendet. Hierbei beachten wir, dass die Gesetze zum Datenschutz (Bundesdatenschutzgesetzes § 40) eingehalten werden.
Damit wir die Daten verwenden dürfen, müssen wir von Dir und Deinen Eltern die sogenannten „Nutzungsrechte" dafür bekommen. Nur dann dürfen wir die Daten für diese wissenschaftliche Untersuchung verwenden und zusammen mit anderen Wissenschaftlern auswerten.

WAS WÜRDE PASSIEREN, WENN ICH DOCH NICHT MEHR AN DER STUDIE TEILNEHMEN WILL?

Die Teilnahme an dieser Studie ist völlig freiwillig. Du kannst jederzeit und ohne sagen zu müssen, warum, die Studie abbrechen. Wir werden dann alle Daten und Blutproben von Dir sofort vernichten. Ansonsten werden wir die Daten aufheben solange sie uns in der Forschung weiterhelfen.

AN WEN KANN ICH MICH BEI FRAGEN WENDEN?

Wenn Du Fragen hast kannst Du Dich an die Ansprechpartner der Abteilung (Telefonnummer auf Seite 1) oder die Ärztin / der Arzt, der Dir die Studie erklärt hat, wenden. Eine **Kopie dieser Information** hast Du erhalten.

Vielen Dank für Dein Interesse und Deine Teilnahme!

Einverständniserklärung zur Datenerhebung im Rahmen der Studie „GENETISCHE EINFLUSSFAKTOREN AUF DIE FURCHTKONDITIONIERUNG UND –GENERALISIERUNG BEI ANGSTSTÖRUNGEN“

Durch meine Unterschrift bestätige ich:

Man hat mir erklärt, dass bei dieser Studie verschiedene Fragen gestellt werden, ich an einem Computertest mitmache, gemessen wird, wie mein Körper in bestimmten Situationen reagiert und dass mir Blut abgenommen wird.

Damit bin ich einverstanden.

Ich habe mir lange genug überlegt, ob ich mitmachen will und durfte Fragen stellen, wenn ich etwas nicht verstanden habe. Ich weiß, dass ich immer die Studie abbrechen darf.

Ich bin einverstanden damit, dass die Informationen in dieser Studie ohne meinen Namen aufgezeichnet, in Computern gespeichert und ausgewertet werden.
Ich bin auch damit einverstanden, dass die Ergebnisse der Studie wissenschaftlich veröffentlicht werden.

Name und Unterschrift des teilnehmenden Kindes:

.....	.....	.....
Name	Datum	Unterschrift

Name und Unterschrift des aufklärenden Mitarbeiters:

.....	.....	.....
Name	Datum	Unterschrift

8.2.2. Informed Consent Form and Sign-up Sheet for Parents

AUFKLÄRUNG UND EINVERSTÄNDNISERKLÄRUNG ZUR TEILNAHME AN DER UNTERSUCHUNG

GENETISCHE EINFLUSSFAKTOREN AUF DIE FURCHTKONDITIONIERUNG UND –GENERALISIERUNG BEI ANGSTSTÖRUNGEN

(FÜR ELTERN VON TEILNEHMENDEN KINDERN)

SINN UND ZWECK DER UNTERSUCHUNG

In unserem Krankenhaus behandeln und erforschen wir Erkrankungen von Kindern und Jugendlichen, die für die Betroffenen und ihre Familien oft sehr belastend und im Alltagsleben einschränkend sind. Angsterkrankungen gehören zu den häufigsten psychischen Störungen im Kindes- und Jugendalter und führen oftmals im Erwachsenenalter zu depressiven Erkrankungen. Heute wissen wir, dass für die Entstehung von Angsterkrankungen sowohl genetische Faktoren als auch Lernerfahrungen eine Rolle spielen. Mit Hilfe der medizinisch-genetischen Erforschung dieser Ursachen versuchen wir, Einblicke in die Entstehung und den Verlauf der Erkrankung zu erhalten und so zu der Entwicklung einer effizienteren Therapie und Prävention beizutragen. Mit den Messungen speziell dieser Studie soll der Einfluss von genetischen Faktoren auf mögliche Entstehungsmechanismen bei Ängsten untersucht werden. Dadurch möchten wir weitere Erkenntnisse über Verarbeitungsprozesse des Gehirns gewinnen und auf lange Sicht die biologischen Grundlagen menschlichen Verhaltens besser verstehen.

WELCHE UNTERSUCHUNGEN SOLLEN DURCHGEFÜHRT WERDEN, WENN ICH EINER TEILNAHME MEINES KINDES AN DER STUDIE ZUSTIMME?

Zuerst möchten wir mit Ihnen und Ihrem Kind eine Befragung zur Erfassung von Symptomen psychischer Erkrankungen durchführen um auszuschließen, dass bei Ihrem Kind eine unentdeckte Erkrankung (insbesondere eine Angststörung) vorliegt. Ihr Kind wird gebeten, Fragen zu Ängstlichkeit und Stimmung zu beantworten. Während der Untersuchung soll es dann Fotographien betrachten, die über einen Computerbildschirm präsentiert werden. In regelmäßigen Abständen wird Ihr Kind zu den Bildern befragt. Diese zeigen 2 weibliche Personen mit neutralem Gesichtsausdruck. Ab und an wird zusätzlich ein ängstlicher Gesichtsausdruck gezeigt. In bestimmten Abständen wird über einen Kopfhörer ein unangenehmes lautes Geräusch dargeboten. Dieses kann einen Augenblick lang unangenehme Gefühle sowie Erregungsgefühle auslösen, ist jedoch weder körperlich noch psychologisch für Ihr Kind gefährlich. Während der Untersuchung möchten wir kontinuierlich Ihre Hautleitfähigkeit und Herzrate als physiologische Maße erheben. Die Messung der

Hautleitfähigkeit erfolgt über 2 kleine Klebeelektroden an der linken Hand, die Herzrate wird über 3 EKG-Elektroden auf dem Brustkorb abgeleitet. Die Untersuchung dauert insgesamt nicht mehr als 120 Minuten.

Um die genetischen Variationen untersuchen zu können, benötigen wir von Ihrem Kind eine geringe Menge Blut (ca. 18 ml). Die Blutentnahme erfolgt unter sterilen Bedingungen, wie sie auch der Hausarzt bei einer Routineuntersuchung durchführt. Die Blutentnahme erfolgt durch medizinisch qualifiziertes Personal (Ärzte, oder durch sie supervidierte Medizin-Doktoranden). Durch spezielle Labormethoden (z.B. Anlage permanenter Zelllinien) ist es möglich, die Blutzellen zu vermehren und so wiederholt zu untersuchen, ohne dass erneut Blut abgenommen werden muss. Die Zellen werden tiefgefroren und in flüssigem Stickstoff aufbewahrt. Bei Bedarf werden die Zellen aufgetaut und stehen dann zur erneuten Untersuchung, z.B. neu bekannt gewordener Genvarianten, zur Verfügung. Die Untersuchungen im Labor der Klinik und Poliklinik für Psychiatrie, Psychosomatik und Psychotherapie oder eines beauftragten Labors sind ausschließlich zur Feststellung von Genvarianten bestimmt. Die Ergebnisse werden nur für diese Studie verwendet.

WAS SIND DIE VORTEILE FÜR MEIN KIND, WENN ES AN DIESER STUDIE TEILNIMMT?

Diese Untersuchung wird keinen direkten Nutzen für Ihr Kind haben, da weder individuelle Ergebnisse weitergegeben noch Sie einen finanziellen Gewinn haben werden. Durch Ihre Bereitschaft, an dieser Studie teilzunehmen, leistet Ihre Familie jedoch einen sehr wichtigen Beitrag zu einem besseren Verständnis psychischer Funktionen und Erkrankungen. Auch wenn wir nicht davon ausgehen können, dass die Ergebnisse in kürzester Zeit zur Entwicklung von neuen Therapien (medikamentös und psychotherapeutisch) führen, erhoffen wir uns erhebliche Vorteile für viele Patienten mit Angststörungen, da wir nur durch die Aufklärung der Entstehungswege von Ängsten Fortschritte in der Therapie erzielen können.

ERGEBEN SICH IRGENDWELCHE RISIKEN FÜR MEIN KIND?

Die Risiken der Blutentnahme sind identisch mit denen einer Routineblutabnahme: lokale Infektion („bakterielle Entzündung, Vereiterung“) und Fehlpunktion einer Schlagader. Beide Risiken sind bei sachgemäßer Durchführung extrem selten.

Wir möchten Sie darauf hinweisen, dass die durchgeführten Untersuchungen ausschließlich Forschungszwecken dienen und keine umfassende Diagnostik körperlicher und psychiatrischer Erkrankungen stattfindet. Im Einzelfall bedeutet dies, dass eventuell vorhandene krankhafte Veränderungen (z. B. im Blut) bei der Untersuchung nicht auffallen. Sollten wir dennoch auf unerwartete Befunde stoßen, die wir für die körperliche oder seelische Gesundheit Ihres Kindes als relevant erachten, werden wir Sie hierüber informieren und das weitere Vorgehen mit Ihnen und gegebenenfalls mit dem behandelnden Arzt besprechen.

WERDEN DIE DATEN VERTRAULICH BEHANDELT?

Wir unterliegen der Schweigepflicht. Alle Informationen, die wir von Ihrer Familie bekommen, werden streng vertraulich behandelt. Alle persönlichen Daten wie z. B. Name und Adresse werden streng getrennt von den Fragebögen, den Interviewdaten, den Ergebnissen der Blutuntersuchungen und der genetischen Tests aufbewahrt. Es werden alle technischen und organisatorischen Maßnahmen getroffen, damit keine Unbefugten an persönliche Informationen gelangen können. Alle medizinischen Informationen werden vor der Verwendung für wissenschaftliche Analysen pseudonymisiert (Fachbegriff: „Pseudonymisierung" gemäß § 3 Abs, 6a Bundesdatenschutzgesetz). Dies bedeutet, dass die Fragebögen, die Aufzeichnungen aus dem Interview, die Blutprobe und die genetischen Testergebnisse mit einem speziellen Zahlencode versehen werden, wenn sie zur Analyse geschickt werden. Persönliche Daten werden den Wissenschaftlern, die die wissenschaftlichen Analysen ausführen, nicht offengelegt und ein direkter Rückgriff auf Ihre Person ist somit ausgeschlossen. Bei allen Datenanalysen sowie beim Austausch von Daten mit kooperierenden Forschergruppen ist der Datenschutz entsprechend des Bundesdatenschutzgesetzes § 40 auf jeden Fall gewährleistet.

ZUSAMMENARBEIT MIT ANDEREN FORSCHUNGSGRUPPEN

In der heutigen Forschung ist eine enge Zusammenarbeit mit anderen wissenschaftlichen Arbeitsgruppen von essentieller Bedeutung, nicht zuletzt da sehr große Probandenkollektive für entsprechende Untersuchungen benötigt werden. Sollte Ihr Kind in unserer Klinik z.B. an anderen Untersuchungen teilnehmen, in denen z. B. Veränderungen im Aufbau oder der Funktionsweise des Gehirns, neuropsychologische Funktionen, somatische Daten oder das Ansprechen auf Medikamente analysiert werden, möchten wir auch diese Befunde hinsichtlich genetischer Zusammenhänge untersuchen. Dies bedeutet, dass hierfür sowohl Biomaterialien als auch Information über klinische Daten zwischen den einzelnen Arbeitsgruppen ausgetauscht werden müssen. Dies geschieht immer gemäß den gesetzlichen Datenschutzrichtlinien und unter Wahrung der Pseudonymisierung.

ÜBERTRAGUNG VON NUTZUNGSRECHTEN

Es ist notwendig, dass Sie uns mit dem Unterschreiben der Einverständniserklärung die Nutzungsrechte Ihrer Daten übertragen. Dies bedeutet, dass wir Ihre Daten im Rahmen der hier beschriebenen Untersuchungsziele nutzen und in pseudonymisierter Form an Dritte (z.B. Kooperationspartner) weitergeben können. Mit der Übertragung der Nutzungsrechte sind jegliche finanziellen Ansprüche ausgeschlossen.

WAS WÜRDE PASSIEREN, WENN ICH ODER MEINE KIND DIE STUDIE ABBRECHEN WOLLTE?

Die Teilnahme an dieser Studie ist absolut freiwillig. Sie und Ihr Kind können selbstverständlich jederzeit und natürlich auch ohne Angabe von Gründen von der Teilnahme zurücktreten. Wir werden dann alle Studienunterlagen und Blutproben von Ihnen sofort vernichten. Ausgenommen hiervon sind Daten, die bereits analysiert wurden und in Publikationen oder Patententwicklungen Eingang gefunden haben.

Für den Fall, dass Sie Ihre Einwilligung nicht zurückziehen, werden alle Informationen und die DNS so lange aufbewahrt und analysiert, wie sie einen wertvollen Beitrag für die Erforschung der biologischen und umweltbedingten Grundlagen psychischer und neurologischer Erkrankungen liefern. Wir werden Ihre Daten also erst dann vernichten, wenn sie nicht mehr zu einem weiteren Wissensgewinn in diesem Forschungsbereich beitragen können.

AN WEN KANN ICH MICH BEI FRAGEN WENDEN?

Bei Rückfragen stehen Ihnen die Ansprechpartner der Abteilung (siehe Seite 1) oder die aufklärende Ärztin / der aufklärende Arzt gerne zur Verfügung. Eine **Kopie dieser Information** wird Ihnen ausgehändigt.

Vielen Dank für Ihr Interesse und Ihre Teilnahme!

Einverständniserklärung zur Datenerhebung im Rahmen der Studie „GENETISCHE EINFLUSSFAKTOREN AUF DIE FURCHTKONDITIONIERUNG UND –GENERALISIERUNG BEI ANGSTSTÖRUNGEN“

Durch meine Unterschrift bestätige ich:

Ich bin darüber informiert worden, dass im Rahmen der o.g. Studie Daten über bestimmte Meinungen und Überzeugungen, sowie physiologische Reaktionen meines Kindes erhoben werden.

Ich erkläre mich freiwillig mit der Datenerhebung einverstanden. Über mögliche Risiken wurde ich aufgeklärt. Ich weiß, dass es nicht möglich ist, Informationen über individuelle Untersuchungsergebnisse zu erhalten.

Ich hatte ausreichend Zeit, mir zu überlegen, ob ich der Datenerhebung zustimmen will, sowie die Gelegenheit, Fragen zu stellen. Mit den erhaltenen Antworten bin ich zufrieden. Ich wurde darauf hingewiesen, dass ich jederzeit von dieser Untersuchung zurücktreten kann, ohne dass mir oder meinem Kind dadurch ein Nachteil entsteht. Die Daten werden in diesem Falle vernichtet.

Ich erkläre mich darüber hinaus damit einverstanden, dass die aus der Datenerhebung gewonnenen Informationen verschlüsselt, d.h., in unpersönlicher Form (ohne Namens- oder Initialennennung) aufgezeichnet, in Computern gespeichert und ausgewertet werden. Dabei gibt es keine Möglichkeit des Rückschlusses auf Einzelpersonen.

Ich bin auch damit einverstanden, dass die Ergebnisse der Studie in Gruppen zusammengefasst wissenschaftlich veröffentlicht werden.

Name des teilnehmenden Kindes: .....

Name und Unterschrift der Erziehungsberechtigten:

1)	.....	.....	.....
	Name	Datum	Unterschrift
2)	.....	.....	.....
	Name	Datum	Unterschrift

Name und Unterschrift des aufklärenden Mitarbeiters:

.....	.....	.....
Name	Datum	Unterschrift

8.3. Informed Consent Form and Sign-up Sheet of Study 3

8.3.1. Informed Consent Form and Sign-up Sheet for Children

Studieninformation im Rahmen der Studie

„Furchtkonditionierung und -generalisierung bei Kindern - eine MR-Bildgebungsstudie oder die Suche nach neuronalen Unterschieden bei Kindern mit unterschiedlich hohen Werten in einem angstrelevanten Fragebogen“

(Für teilnehmende Kinder)

Liebe/r Studienteilnehmer/in,

Viele Kinder, die zu uns ins Krankenhaus kommen, haben Angst vor bestimmten Situationen und sind dadurch sehr belastet. Manche trauen sich nicht vor anderen zu sprechen oder haben große Angst vor Hunden; wieder andere haben so viel Angst, dass sie nicht mehr in die Schule gehen wollen. Wir sind Wissenschaftler an der Universitätsklinik und wir wissen bereits, dass bei vielen Kindern Ängste mit der Zeit eher zunehmen. Nun möchten wir herausfinden, wie diese Ängste entstehen und wie Angst im Gehirn aussehen kann. Dadurch wollen wir neue Ideen sammeln, wie wir diese Ängste besser behandeln können. Dabei kannst Du uns helfen!

Wie läuft die Untersuchung ab?

Während der Untersuchung liegst Du für ca. 30 min mit dem Rücken in einer Röhre. Das ist der sogenannte MR-Tomograph, der mit Hilfe von Magnetwellen Bilder von deinem Gehirn macht. Währenddessen schaust Du dir Fotografien von Gesichtern an. Manchmal hörst Du dann auch über einen Kopfhörer ein unangenehmes lautes Geräusch. Das ist zwar kurz unangenehm, aber gar nicht gefährlich.

Kann mir bei der MRT-Untersuchung etwas passieren?

Vor Beginn der Untersuchung erklären wir Dir genau, was passieren wird, und Du hast Gelegenheit Dir das Gerät anzuschauen und Fragen zu stellen. Dann kannst Du Dich hinlegen und wir schieben Dich mit dem Kopf voran in das Gerät, das aussieht wie eine große Röhre. Darin ist es eng, was manche Kinder nicht mögen, aber wenn Du Angst bekommst kannst Du das immer sagen, weil wir Dich über ein Mikrophon immer hören können! Wenn die Bilder gemacht werden, macht das Gerät ein lautes Klopfgeräusch. Das ist nicht gefährlich, aber um deine Ohren zu schützen musst Du Ohrstöpsel sowie einen Kopfhörer tragen. Bei solchen Untersuchungen, wie wir sie mit Dir machen wollen, ist noch nie etwas Gefährliches passiert. Um mitmachen zu können, gibt es aber ein paar Bedingungen. Beantworte deswegen bitte die Fragen auf der letzten Seite so gut Du kannst. Wenn Du etwas nicht verstehst, helfen Dir die Untersuchungsleiterin oder deine Eltern.

Bekomme ich die Ergebnisse der Untersuchung mitgeteilt?

Alles was wir über Dich erfahren, benutzen wir nur zur Forschung, Wenn uns aber etwas auffällt, was auf eine Krankheit hinweisen könnte, sagen wir Dir und Deinen Eltern Bescheid wenn Ihr das wollt und besprechen mit Euch, was weiter zu tun ist.

Kann ich meine Einwilligung zurücknehmen?

Die Teilnahme an dieser Studie ist völlig freiwillig. Wenn Du nicht mehr bei der Untersuchung mitmachen möchtest, kannst Du das der Untersuchungsleiterin sagen und jederzeit aufhören. Du musst uns keinen Grund sagen und hast auch keine Nachteile deswegen. Wenn Du das willst, vernichten wir alle deine Daten.

Es bestehen keine ethischen Bedenken von Seiten der Ethikkommission der Medizinischen Fakultät der Universität Würzburg gegen die Durchführung der Studie.

Die Verantwortung während der gesamten Studie trägt Prof. Dr. Marcel Romanos (Klinik für Kinder- und Jugendpsychiatrie, Psychosomatik und Psychotherapie, Universitätsklinikum Würzburg, Füchsleinstraße 15, 97080 Würzburg).

An wen kann ich mich bei Fragen wenden?

Bei Rückfragen steht Dir Fr. Julia Reinhard (Telefon: 0931 / 201-76911 oder 0151 / 53833792 oder

E-Mail: Reinhard_J@ukw.de) gerne zur Verfügung. Eine Kopie dieser Information wird Dir ausgehändigt.

Vielen Dank für Dein Interesse und Deine Teilnahme!

Datenschutz

Alle Menschen, die Dich untersuchen, habe eine Schweigepflicht, das heißt alles was wir von Dir erfahren behandeln wir geheim. Auf alle Daten schreiben wir statt deinem Namen einen Code, damit niemand gleich sehen kann, von wem sie kommen. Andere Leute bekommen keine Informationen über Dich persönlich. Wenn wir etwas herausfinden, was für alle Kinder gilt, die so ähnlich sind wie Du, dann schreiben wir darüber in einer Zeitung, aber niemand weiß, dass Du bei der Untersuchung mitgemacht hast.

MRT-Fragebogen

Hohe Magnetfelder bewirken, dass metallische Implantate oder Fremdkörper im Körper wandern können, was zu Komplikationen führen kann. Das hohe Magnetfeld des Tomographen bewirkt weiterhin, dass elektronisch aktive Implantate (z.B. Herzschrittmacher) nicht mehr ordnungsgemäß funktionieren, deshalb dürfen Herzschrittmacher-Träger an dieser Studie nicht teilnehmen.

Solltest Du Schrittmacherträger sein, oder sich Metallteile in Deinem Körper befinden, informiere bitte das Untersuchungspersonal darüber.

Bei festen Zahnspangen bzw. Retainern kann es in seltenen Fällen zu Wärmeentwicklung kommen. In diesem Fall wirst Du dazu instruiert, die Messung abzubrechen. Metallteile, die Du mit Dir führst, sind eine potentielle Gefahr für Dich und andere. Elektronische Datenträger und Geräte (z.B. Kredit- und Scheckkarten, Mobiltelefone, Uhren, Hörgeräte) können im Magnetfeld unbrauchbar werden. Bitte lege daher Schlüssel, Geldbeutel, Mobiltelefone und dergleichen vor der Untersuchung ab.

BITTE ALLE FRAGEN BEANTWORTEN	**Ja**	**Nein**
Trägst Du ein aktives Implantat? (z.B. Herzschrittmacher, Neurostimulator, Medikamentenpumpe)		
Bist Du schon einmal operiert worden? (gegebenenfalls wann und wie häufig)		
Befinden sich metallische oder elektronische Teile an oder in Deinem Körper? (z.B. Splitter, Prothesen, Metallplatten, Klammern, Zahnspangen, Spirale, Piercing)		
Bist Du tätowiert?		
Hast oder hattest Du mit Metallverarbeitung zu tun?		
Leidest Du unter Angst vor engen Räumen oder Platzangst?		
Hattest Du schon einmal epileptische Anfälle? (wenn ja, wann und wie häufig)		
Nimmst Du momentan Medikamente? (wenn ja, bitte auflisten mit Dosierung)		
Wie ist Dein aktuelles Gewicht?		**kg**
Wie ist Deine aktuelle Größe?		**cm**

Einwilligungserklärung zur Studie

„Furchtkonditionierung und -generalisierung bei Kindern -

eine MR-Bildgebungsstudie oder die Suche nach neuronalen Unterschieden bei Kindern mit unterschiedlich hohen Werten in einem angstrelevanten Fragebogen“

(Für teilnehmende Kinder)

Durch meine Unterschrift bestätige ich:

Man hat mir erklärt, dass ich bei dieser Untersuchung für ca. 30 min auf dem Rücken in einer Röhre liegen werde. Mir werden Gesichter gezeigt. Manchmal werde ich auch ein unangenehmes lautes Geräusch hören, was unangenehm sein kann. Damit bin ich einverstanden.

Ich habe mir lange genug überlegt, ob ich mitmachen will und durfte Fragen stellen, wenn ich etwas nicht verstanden habe. Ich weiß, dass ich dem Untersuchungsleiter jederzeit sagen kann, wenn ich nicht mehr mitmachen möchte. Ich muss dafür keinen Grund sagen und habe auch keine Nachteile deswegen.

Ich bin einverstanden damit, dass die Informationen in dieser Studie ohne meinen Namen aufgezeichnet, in Computern gespeichert und ausgewertet werden. Ich bin auch damit einverstanden, dass die Ergebnisse der Studie in einer Zeitschrift veröffentlicht werden.

Name des teilnehmenden Kindes: .....

Name und Unterschrift der Erziehungsberechtigten:

3)	.....	.....
Name	Datum	Unterschrift
4)	.....	.....
Name	Datum	Unterschrift

Name und Unterschrift des aufklärenden Mitarbeiters:

.....	.....	.....
Name	Datum	Unterschrift

8.3.2. Informed Consent Form and Sign-up Sheet for Parents

Studieninformation im Rahmen der Studie

„Furchtkonditionierung und -generalisierung bei Kindern - eine MR-Bildgebungsstudie oder die Suche nach neuronalen Unterschieden bei Kindern mit unterschiedlich hohen Werten in einem angstrelevanten Fragebogen“

(Für Eltern/Sorgeberechtigte von minderjährigen Studienteilnehmern)

Liebe Eltern, liebe Sorgeberechtigte,

vielen Dank für Ihr Interesse an unserer Studie. Angsterkrankungen gehören zu den häufigsten psychischen Erkrankungen bei Kindern und führen oft zu Depressionen im Erwachsenenalter. Wir wissen, dass Furchtgeneralisierung ein wichtiger Faktor in der Entstehung von Angsterkrankungen ist. Das bedeutet, dass man nicht nur vor einem bestimmten Objekt Angst hat, sondern auch vor Stimuli, die diesem mehr oder weniger ähnlich sind. Menschen, die zu Angsterkrankungen neigen, generalisieren stärker als gesunde Personen. Uns interessiert, wie sich Unterschiede in der Furchtgeneralisierung im Gehirn widerspiegeln. Ziel ist es, zu klären, wie wir Kinder besser vor Angsterkrankungen und deren Folgen schützen können. Durch Ihre Bereitschaft, an dieser Studie teilzunehmen, leistet Ihre Familie einen sehr wichtigen Beitrag zu einem besseren Verständnis psychischer Funktionen und Erkrankungen. Auch wenn wir nicht davon ausgehen können, dass die Ergebnisse in kürzester Zeit zur Entwicklung von neuen Therapien (medikamentös und psychotherapeutisch) führen, erhoffen wir uns erhebliche Vorteile für viele Patienten mit Angststörungen, da wir nur durch die Aufklärung der Entstehungswege von Ängsten Fortschritte in der Therapie erzielen können.

Wie läuft die Untersuchung ab?

Während der MRT- Untersuchung liegt Ihr Kind für ca. 30 min in einer Röhre, dem sogenannten MR-Tomographen. Währenddessen werden Ihrem Kind Fotografien von Gesichtern gezeigt. Diese zeigen 2 weibliche Personen mit neutralem Gesichtsausdruck. In bestimmten Abständen wird ein lautes Geräusch dargeboten. Dieses kann einen Augenblick lang unangenehme Gefühle sowie Erregungsgefühle auslösen, ist jedoch weder körperlich noch psychologisch für Ihr Kind gefährlich.

Welche Risiken sind mit der MRT-Untersuchung verbunden?

Die Untersuchungsmethode MRT ist als Routineuntersuchung etabliert. Vor Beginn der Untersuchung erklären wir Ihnen / Ihrem Kind genau den Ablauf und es hat ausreichend Gelegenheit, sich mit dem Gerät vertraut zu machen. Dann wird Ihr Kind auf einer speziellen Liege mit dem Kopf voran in das MRT-Gerät gefahren. Dabei handelt es sich um ein röhrenförmiges Gebilde, in dem es auf dem Rücken liegen wird. Über eine Sprechanlage und ein Mikrophon steht Ihr Kind mit uns in ständiger Verbindung. Während der Zeit in der Röhre, die im Normalfall ca. 30 Minuten dauert, werden Bilder von dem Gehirn

Ihres Kindes aufgenommen. Weil dabei ein lautes Klopfen und Lärm im Gerät entsteht, muss es sie einen Hörschutz tragen. Da die Röhre sehr wenig Raum bietet, kann es vorkommen, dass bei manchen Probanden durch die Enge Platzangst entsteht. Sollte dies bei Ihrem Kind der Fall sein, kann Ihr Kind des sogenannten ‚Notfallball' drücken, der während der Untersuchung im Schoß Ihres Kindes liegt. Dadurch wird ein Signalton bei den Untersuchern ausgelöst und die Untersuchung wird abgebrochen. Außerdem ist es Ihrem Kind zu jedem Zeitpunkt möglich, das dem Untersucher über die Sprechanlage mitzuteilen und den Versuch gegebenenfalls abzubrechen.
Diese Untersuchungen sind nicht invasiv und beeinträchtigen Ihr Kind nach heutigem Kenntnisstand nicht. Um an der Studie teilnehmen zu können, müssen bestimmte Voraussetzungen gegeben sein. Füllen Sie deshalb bitte den beigelegten Fragebogen sorgfältig aus (s. u.).
Die im Rahmen der MRT Studie erhobenen Datensätze dienen ausschließlich wissenschaftlichen Zwecken und sind **nicht** für das Ausstellen klinischer Diagnosen optimiert. Trotzdem besteht die Möglichkeit, dass eine Unregelmäßigkeit (ein sog. **Zufallsbefund**) in dem Gehirn Ihres Kindes festgestellt wird, was manchmal auf eine Erkrankung hinweisen könnte. Bei Entdeckung eines solchen Zufallsbefundes wird Ihnen - sofern Sie es wünschen und in der Einverständniserklärung entsprechend vermerkt haben - dieser Befund in einem persönlichen Gespräch mitgeteilt und der Kontakt zu einem entsprechenden Spezialisten hergestellt.

Erfolgt eine Rückmeldung über die Ergebnisse der Datenerhebung?
Die Datenerhebung erfolgt für rein wissenschaftliche Zwecke. Individuelle Untersuchungsergebnisse werden im Regelfall nicht rückgemeldet.

Kann ich meine Einwilligung widerrufen?
Die Teilnahme an der Studie ist völlig freiwillig. Sie können jederzeit, ohne Angabe von Gründen und ohne Nachteile für Sie oder Ihr Kind von der Studienteilnahme zurücktreten. In diesem Fall werden bereits gewonnene Informationen ausgewertet, oder auf Ihren ausdrücklichen Wunsch hin gelöscht.

Es bestehen keine ethischen Bedenken von Seiten der Ethikkommission der Medizinischen Fakultät der Universität Würzburg gegen die Durchführung der Studie. Die Verantwortung während der gesamten Studie trägt Prof. Dr. Marcel Romanos (Klinik für Kinder- und Jugendpsychiatrie, Psychosomatik und Psychotherapie
Universitätsklinikum Würzburg, Füchsleinstraße 15, 97080 Würzburg).

An wen kann ich mich bei Fragen wenden?
Bei Rückfragen steht Ihnen Fr. Julia Reinhard (Telefon: 0931 / 201-76911 oder 0151 / 53833792 oder E-Mail: Reinhard_J@ukw.de) gerne zur Verfügung. Eine Kopie dieser Information wird Ihnen ausgehändigt.

Vielen Dank für Ihr Interesse und Ihre Teilnahme!

Datenschutz

Für uns gilt die ärztliche Schweigepflicht. Wie alle anderen Informationen, die wir von Ihnen und Ihrem Kind erhalten, unterliegen auch die Informationen aus der Datenerhebung dem Datenschutz. Das bedeutet, dass erhobene Daten in pseudonymisierter Form - also ohne Nennung des Namens Ihres Kindes - für einen Zeitraum von 10 Jahre elektronisch gespeichert werden. Der Zugriff auf alle Daten ist autorisierten Personen vorbehalten, die direkt mit der Untersuchung im Zusammenhang stehen. Die erhobenen Daten dienen rein wissenschaftlichen Zwecken und werden ohne Bezug auf konkrete Personen ausgewertet und in wissenschaftlichen Fachzeitschriften veröffentlicht.

MRT-Fragebogen

Hohe Magnetfelder bewirken, dass metallische Implantate oder Fremdkörper im Körper wandern können, was zu Komplikationen führen kann. Das hohe Magnetfeld des Tomographen bewirkt weiterhin, dass elektronisch aktive Implantate (z.B. Herzschrittmacher) nicht mehr ordnungsgemäß funktionieren, deshalb dürfen Herzschrittmacher-Träger an dieser Studie nicht teilnehmen. Sollte Ihr Kind Schrittmacherträger sein, oder sich Metallteile im Körper Ihres Kindes befinden, informieren Sie bitte das Untersuchungspersonal darüber.

Bei festen Zahnspangen bzw. Retainern kann es in seltenen Fällen zu Wärmeentwicklung kommen. In diesem Fall wird Ihr Kind dazu instruiert, die Messung abzubrechen.

Metallteile, die Ihr Kind mit sich führt, sind eine potentielle Gefahr für Ihr Kind und Andere. Elektronische Datenträger und Geräte (z.B. Kredit- und Scheckkarten, Mobiltelefone, Uhren, Hörgeräte) können im Magnetfeld unbrauchbar werden. Bitte nehmen Sie Schlüssel, Geldbeutel, Mobiltelefone und dergleichen Ihres Kindes vor der Untersuchung an sich.

BITTE ALLE FRAGEN BEANTWORTEN	**Ja**	**Nein**
Trägt Ihr Kind ein aktives Implantat? (z.B. Herzschrittmacher, Neurostimulator, Medikamentenpumpe)		
Ist Ihr Kind schon einmal operiert worden? (gegebenenfalls wann und wie häufig)		
Befinden sich metallische oder elektronische Teile am oder im Körper Ihres Kindes? (z.B. Splitter, Prothesen, Metallplatten, Klammern, Zahnspangen, Spirale, Piercing)		
Ist Ihr Kind tätowiert?		
Hat oder hatte Ihr Kind mit Metallverarbeitung zu tun?		
Leidet Ihr Kind unter Angst vor engen Räumen oder Platzangst?		
Hatte Ihr Kind schon einmal epileptische Anfälle? (wenn ja, wann und wie häufig)		
Nimmt Ihr Kind momentan Medikamente? (wenn ja, bitte auflisten mit Dosierung)		
Wie ist das aktuelle Gewicht Ihres Kindes?		**kg**
Wie ist die aktuelle Größe Ihres Kindes?		**cm**

Einwilligungserklärung zur Studie

„Furchtkonditionierung und -generalisierung bei Kindern - eine MR-Bildgebungsstudie oder die Suche nach neuronalen Unterschieden bei Kindern mit unterschiedlich hohen Werten in einem angstrelevanten Fragebogen“

(Für Eltern/Sorgeberechtigte von minderjährigen Studienteilnehmern)

Durch meine Unterschrift bestätige ich:

Die vorgesehene Untersuchung einschließlich der Risiken, sowie die Studieninformation und die Einverständniserklärung wurden mir durch Herrn / Frau _____ zu meiner Zufriedenheit erklärt. Alle meine Fragen wurden so umfassend beantwortet, dass ich gut darüber informiert bin, warum diese Untersuchung durchgeführt wird und wie die Studie aufgebaut ist.

Ich habe die Studieninformation gelesen und verstanden. Ich konnte die schriftliche Probandeninformation behalten und habe eine Kopie der schriftlichen Einverständniserklärung erhalten. Ich hatte genügend Zeit, um eine Entscheidung zu treffen, und bin einverstanden, dass mein Kind an der Messung im Magnetresonanztomographen (ca. 30 Minuten) teilnimmt.

Ich erkläre mich freiwillig mit der Datenerhebung einverstanden. Über mögliche Risiken wurde ich aufgeklärt. Ich weiß, dass es mir nicht möglich ist, Informationen über die Untersuchungsergebnisse meines Kindes zu erhalten. Die Teilnahme meines Kindes ist freiwillig. Wenn es die Teilnahme ablehnt oder widerruft, wird dies keinerlei Nachteile zur Folge haben. Namen und Daten werden streng vertraulich behandelt und ausgewertet, ohne den Namen meines Kindes zu nennen.

Ich erkläre mich darüber hinaus damit einverstanden, dass die aus der Datenerhebung gewonnenen Informationen gemäß geltender Datenschutzbestimmungen verschlüsselt (d.h., in unpersönlicher Form ohne Namens- oder Initialen-Nennung) aufgezeichnet, in Computern gespeichert und ausgewertet werden. Ich bin auch damit einverstanden, dass die Ergebnisse der Studie in Gruppen zusammengefasst wissenschaftlich veröffentlicht werden.

Es ist mir jederzeit möglich, die Einwilligung zur Teilnahme ohne Angabe von Gründen zurückzuziehen, ohne Gründe zu nennen. Hieraus werden meinem Kind keinerlei Nachteile entstehen. Die bis dahin gewonnenen Daten werden auf meinen Wunsch hin vernichtet und fachgerecht entsorgt.

Über Zufallsbefunde mit möglichem Krankheitswert im Rahmen der MRT-Untersuchung möchte ich informiert werden.

Ja	Nein
☐	☐

Name des teilnehmenden Kindes: .....

Name und Unterschrift der Erziehungsberechtigten:

5)	.....	.....	.....
	Name	Datum	Unterschrift
6)	.....	.....	.....
	Name	Datum	Unterschrift

Name und Unterschrift des aufklärenden Mitarbeiters:

.....	.....	.....
Name	Datum	Unterschrift

8.4. Questionnaires

8.4.1. STAIC-Trait

STAIC

Im folgenden Fragebogen findest Du eine Reihe von Feststellungen, die Mädchen und Jungen benutzen, um sich selbst zu beschreiben. Lies Dir jede Feststellung durch und entscheiden Sie, ob sie fast nie, oder manchmal oder oft auf Dich zutrifft. Kreuze dann bitte für jede Feststellung das entsprechende Kästchen an.

Es gibt keine richtigen oder falschen Antworten. Überlege bitte nicht zu lange und denke daran, diejenige Antwort auszuwählen, die am besten beschreibt, wie Du Dich im Allgemeinen fühlst.

1. Ich habe Angst, Fehler zu machen	☐ fast nie	☐ manchmal	☐ oft
2. Mir ist zum weinen zumute	☐ fast nie	☐ manchmal	☐ oft
3. Ich fühle mich unglücklich	☐ fast nie	☐ manchmal	☐ oft
4. Es fällt mir schwer, mich zu entscheiden	☐ fast nie	☐ manchmal	☐ oft
5. Es fällt mir schwer, meine Probleme anzupacken	☐ fast nie	☐ manchmal	☐ oft
6. Ich mache mir zuviel Sorgen	☐ fast nie	☐ manchmal	☐ oft
7. Zu Hause rege ich mich auf	☐ fast nie	☐ manchmal	☐ oft
8. Ich bin schüchtern	☐ fast nie	☐ manchmal	☐ oft
9. Ich bin beunruhigt	☐ fast nie	☐ manchmal	☐ oft
10. Unwichtige Gedanken gehen mir durch den Kopf und stören mich	☐ fast nie	☐ manchmal	☐ oft
11. Ich mache mir Sorgen über die Schule	☐ fast nie	☐ manchmal	☐ oft
12. Ich habe Schwierigkeiten zu entscheiden, was ich tun soll	☐ fast nie	☐ manchmal	☐ oft
13. Ich merke, dass mein Herz schneller schlägt	☐ fast nie	☐ manchmal	☐ oft
14. Ich fürchte mich heimlich	☐ fast nie	☐ manchmal	☐ oft
15. Ich mache mir Sorgen um meine Eltern	☐ fast nie	☐ manchmal	☐ oft
16. Ich bekomme feuchte Hände	☐ fast nie	☐ manchmal	☐ oft
17. Ich mache mir Sorgen über Dinge, die passieren könnten	☐ fast nie	☐ manchmal	☐ oft
18. Es fällt mir schwer, abends einzuschlafen	☐ fast nie	☐ manchmal	☐ oft
19. Ich habe ein komisches Gefühl im Magen	☐ fast nie	☐ manchmal	☐ oft
20. Ich grübele darüber nach, was andere Personen von mir denken	☐ fast nie	☐ manchmal	☐ oft

8.4.2. The Edinburgh Inventory of Handedness

VpNr.: _____

EIOH (Edinburgh Inventory of Handedness)

Bitte dokumentieren Sie die Handpräferenz Ihres Kindes für die unten aufgelisteten Handlungen oder Objekte durch ein „+“ in dem dafür vorgesehenen Kästchen. Ist die Bevorzugung einer Hand bei einer Tätigkeit/einem Objekt so stark, dass Ihr Kind niemals versuchen würden, die andere dafür zu verwenden, dann fügen Sie ein „++“ ein. Sind Sie bei einer Antwort unentschieden, dann fügen Sie in beiden Kästchen ein „+“ ein.

Für die Ausführung mancher Tätigkeiten oder die Benutzung einiger Objekte sind zwei Hände notwendig. In diesem Fall ist in Klammern angegeben, welcher Teil der Handlung genau gemeint ist.

Bitte beantworten Sie alle Fragen und lassen Sie nur dann eine Lücke, wenn Ihr Kind mit der Tätigkeit/dem Objekt überhaupt keine Erfahrung hat.

		links	**rechts**
1	Schreiben		
2	Zeichnen		
3	Werfen		
4	Schere		
5	Zahnbürste		
6	Messer (ohne Gabel)		
7	Löffel		
8	Schießen beim Ballspielen		
9	Eine Kiste öffnen (Deckel)		

8.5. Publications

8.5.1. Paper

Schiele, M. A.*, **Reinhard, J**.*, Reif, A., Domschke, K., Romanos, M., Deckert, J., & Pauli, P. (2016). Developmental aspects of fear: Comparing the acquisition and generalization of conditioned fear in children and adults. Developmental Psychobiology, 58(4), 471-481.
***equal contribution**

8.5.2. Conference Proceedings

Reinhard, J., Kneer, K., Romanos, M., & Neufang, S. (2017). Trait anxiety modulates fear learning and fear generalization in fronto-limbic pathways of the developing brain. *International Congress of the World Association for Stress Related and Anxiety Disorders (WASAD) in conjunction with the Collaborative Research Center SFB-TRR 58, Fear, Anxiety, Anxiety Disorders*, Würzburg. And: *Second International Summer School: Emotional Learning and Memory in Health and Psychopathology*, Leuven, Belgium.

Reinhard, J., Neufang, S., Schiele, M.A., Reif, A., Domschke, K., Deckert, J., Pauli, P., & Romanos, M. (2017). Children's trait anxiety modulates generalization of conditioned fear. *XXXV. Kongress der Deutschen Gesellschaft für Kinder- und Jugendpsychiatrie, Psychosomatik und Psychotherapie e.V. (DGKJP)*, Ulm.

Reinhard, J., Schiele, M.A., Andreatta, M., Reif, A., Domschke, K., Deckert, J., Pauli, P., & Romanos, M. (2016). Children's trait anxiety modulates generalization of conditioned fear. *Jahrestagung der Gesellschaft für Angstforschung (GAF)*, Tübingen. And: *Wissenschaftskonferenz des Zentrums für Psychische Gesundheit (ZEP)*, Würzburg.

Reinhard, J., Schiele, M.A., Andreatta, M., Reif, A., Domschke, K., Deckert, J., Romanos, M., & Pauli, P., (2016). Children's trait anxiety modulates generalization of conditioned fear. *Jahreskongress der Deutschen Gesellschaft für Biologische Psychiatrie (DGBP)*, Würzburg.

Reinhard, J., Domschke, K., Deckert, J., Reif, A., Pauli, P., & Romanos, M. (2016). Children's trait anxiety modulates generalization of conditioned fear. Effects of trait anxiety on acquisition and generalization of conditioned fear in children. *Forschungstagung der Deutschen Gesellschaft für Kinder- und Jugendpsychiatrie, Psychosomatik und Psychotherapie e.V. (DGKJP)*, Frankfurt.

Reinhard, J., Schiele, M.A., Reif, A., Domschke, K., Deckert, J., Pauli, P., & Romanos, M., (2015/2016). Developmental aspects of fear: Comparing the acquisition and generalization of conditioned fear in children and adults. *Forschungstagung der Deutschen Gesellschaft für Kinder- und Jugendpsychiatrie, Psychosomatik und Psychotherapie e.V. (DGKJP)*, Frankfurt. And: *Seventh European Meeting on Human Fear Conditioning*, Bochum.

Reinhard, J., Schiele, M.A., Deckert, J., Reif, A., Domschke, K., Pauli, P., & Romanos, M. (2015). Fear-Learning and Generalization – A comparison between Adults and Children. *XXXIV. Kongress der Deutschen Gesellschaft für Kinder- und Jugendpsychiatrie, Psychosomatik und Psychotherapie e.V. (DGKJP)*, München.

Reinhard, J., Pauli, P., Deckert, J., Reif, A., & Romanos, M. (2014). Furchtgeneralisierung bei Kindern zwischen 8 und 12 Jahren – Einflussfaktoren auf die Entwicklung von Furcht und Angst. *XXI. Biologische Arbeitskreistagung und VI. Psychotherapietagung der Kinder- und Jugendpsychiatrie, Psychosomatik und Psychotherapie*, Würzburg.

Reinhard, J., Reif, A., Pauli, P., Deckert, J., & Romanos, M. (2014). Fear-Generalization in Children – Influencing Factors on the Development of Fear and Anxiety. *Eureka*, Würzburg.

Schiele, M.A., **Oechsner, J**., Schartner C., Baumann, C. Romanos, M., Pauli, P., Deckert, J., & Reif, A. (2013). Gene-Environment Interactions in Dimensional Endophenotypes of Fear and Anxiety and Their Generalization in Adults and Children. *SFB-TRR 58 Fear, Anxiety, Anxiety Disorders Symposium*, Hamburg