ABSTRACT

Title of Thesis: FEAR CONDITIONING ACROSS DEVELOPMENT: AN EXAMINATION OF FEAR ACQUISITION, EXTINCTION, AND GENERALIZATION IN 5-TO-10 YEAR OLD CHILDREN

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The acquisition and extinction of fear is widely studied using fear conditioning (FC) paradigms. Few studies, however, have examined how fear learning emerges across development. Understanding the developmental mechanisms underlying FC can provide a framework to examine disruptions in fear learning, particularly when fears become pervasive as in the case of anxiety disorders. Traditional FC paradigms in adult and animal studies involve aversive stimuli, like shock, which present ethical limitations in youth. The present study aimed to examine the validity of a novel FC paradigm in a sample of sixty-four typically developing 9-to-15 year old children. Results revealed differential learning to the CS+ compared to the CS- during conditioning as evidenced by subjective fear ratings and greater skin conductance response. No differences emerged across pre-conditioning and extinction phases. Results from this study indicate the utility of this novel bell conditioning paradigm at eliciting fear learning and extinction behaviors in children.

# FEAR CONDITIONING ACROSS DEVELOPMENT: AN EXAMINATION OF FEAR ACQUISITION, EXTINCTION, AND GENERALIZATION IN 5-TO-10 YEAR OLD CHILDREN

by

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CHAPTER 1

## Introduction

Learning to recognize danger facilitates survival, and individual differences in the capacity for such learning may be one source for the emergence of anxiety disorders. Therefore, understanding the mechanisms underlying fear conditioning may shed light on normative and pathological fear responses. Fear responses emerge early in development, and perturbed fear learning may contribute to pediatric anxiety. Hence, a developmental framework may inform understandings of individual differences in fear learning.

The aim of this paper is to review studies of the development of fear conditioning with an emphasis on pediatric populations. Because only a few studies on fear conditioning and extinction in children are available, the current review attempts to extrapolate from animal models and data from human adults. Another important aim is to discuss the methodological and ethical considerations in conducting fear conditioning studies in children and to suggest appropriate methods for future research. This review unfolds in four stages. The first defines the major concepts relevant to fear conditioning and extinction while emphasizing the importance of studying them developmentally. The second reviews the neurobiology of fear conditioning and extinction derived from work in animal models and humans. The third details findings from fear conditioning and extinction studies conducted in children and adolescents. And finally, the fourth reviews common methodology used to encourage improved design to study these processes in children and adolescents and review possible direction for future studies.

## Studying fear conditioning and extinction developmentally

*Fear conditioning*, a form of associative learning, is a widely used experimental paradigm for investigating the psychophysiological processes and neural mechanisms sub-serving learning about danger cues in a range of mammalian species. In classical fear conditioning, a neutral conditioned stimulus (CS, e.g., tone) is repeatedly paired with an aversive stimulus (UCS, e.g., shock), yielding a CS-UCS association. Discrimination conditioning uses two CSs, one that is paired with the UCS (CS+) and another that is not (CS–). A conditioned response (CR, e.g., freezing behavior) is produced in response to the CS+, thus enhancing the organism's ability to respond to similar events in the future. This paradigm allows for the rapid induction of a learned fear state and the expression of learned fear-related behaviors. Conditioned fear responses have been found across multiple species and include various responses such as changes in autonomic activity (e.g. heart rate, blood pressure, skin conductance), defensive behaviors (e.g., freezing), endocrine response (e.g., hormone release), pain sensitivity (e.g., analgesia), and modulation of reflex expressions, like fear potentiated startle and eye blink response (LeDoux, 2000).

*Extinction*, a process that is complementary to fear learning, involves three main phases: acquisition of extinction, consolidation, and retrieval (Quirk & Mueller, 2008). During extinction, the CS+ is presented in the absence of the UCS, leading the conditioned response (CR) to decline across repeated presentations. There is now a growing consensus that extinction does not eradicate the initial CS+-UCS association but rather creates new learning, where the CS+ is associated with the absence of the UCS (for review see (Schiller et al., 2010)). Subsequently, cascades of neural and molecular

processes interact to consolidate a long-term extinction memory. Following successful extinction, the initial CS+-UCS association competes with the newer CS+-no-UCS association. When presentation of the extinguished CS triggers the no-UCS memory, it inhibits the original CR. Nevertheless, evidence of competition of the two memories manifests when conditioned fear responses reappear following contextual manipulations (i.e., renewal) or presentation of the UCS even in the absence of the CS+ (i.e., reinstatement) (Bouton, 2002).

Interactions between fear conditioning and extinction shape behavior, particularly during development, when the effects of learning can be particularly profound. Hence, understanding the developmental trajectories of the two processes and the neural circuitries that support them informs a mechanistic understanding of fear. Fear conditioning emerges early in life, involving subcortical areas predominantly the amygdala, whereas extinction appears to emerge later in development and involve the prefrontal cortex (Kim & Richardson, 2010). Thus, when studying these two learning-related processes, a developmental perspective examining maturation of brain regions supporting fear learning and fear extinction may explain the emergence of individual differences in fear and anxiety.

**The Neural Circuitry Underpinning Fear Conditioning and extinction**

Most neuroscience research on fear conditioning uses animal models. Nonetheless, translating these findings to human studies is feasible due to the strong cross-species similarities in the physiology of fear (LeDoux, 2000). Animal models are particularly important for studying the emergence of fear conditioning across development as some of the procedures are less feasible in humans and particularly in

children and adolescents. Therefore, findings from animal models can be translated to research in human adults, which in turn can be applied to pediatric populations.

### *Animal models*

#### *Fear conditioning*

Fear conditioning involves the processing of sensory information about the CS and the UCS, which occurs in relevant sensory cortices as well as the thalamus and hypothalamus and the brainstem periacqueductal gray region. Typically, the CS and UCS are presented in different sensory modalities (e.g., auditory tone and tactile shock). Ultimately, information about the CS and the paired UCS is thought to first converge in the baso-lateral nucleus of the amygdala. Initially, the neutral CS will produce weaker amygdala stimulation than produced by the UCS. Following CS-UCS pairings, the initially weak amygdala stimulation produced by the CS becomes stronger, reflecting a CS-UCS association. After this association is formed, the weak stimulus, presented on its own without the UCS, has the capacity to elicit a stronger amygdala response, thus influencing behavior and physiology through efferent projections from the central nucleus of the amygdala. This region of the amygdala sends projections to brainstem and motor areas that control the expression of fear responses across a variety of domains expressed via behavioral, autonomic nervous system, and endocrine responses (LeDoux, 2000).

The amygdala appears to enhance learning by influencing cortical plasticity reflecting changes in synaptic connection, particularly during learning. Once a CS-UCS association has been acquired, a decline in amygdala activation may occur (Buchel & Dolan, 2000). However, later-appearing changes in the CS-UCS association may occur

through further changes in amygdala. For example, mounting evidence implicates a portion of the medial prefrontal region (mPFC), the so-called "pre-limbic" cortex, in enhancement of amygdala activity and its importance for expression of conditioned fear. Specifically, it is proposed that this region integrates input from other brain structures to enhance the expression of fear conditioning via excitatory projections to the amygdala (Corcoran & Quirk, 2007; Sierra-Mercado, Padilla-Coreano, & Quirk, 2011; Sotres-Bayon & Quirk, 2010).

Relative to the considerable work on fear conditioning in mature rodents and primates, far less work examines developmental aspects of fear conditioning using developmental animal models (Kim & Richardson, 2010). Research investigating the emergence of fear conditioning in infant rats has identified a sensitive period in which amygdala activation to aversive stimuli is inhibited. During early stages of postnatal development, newborns are equipped with innate abilities for appetitive learning (Landers & Sullivan, 2012). For instance, infant rats acquire the ability to orient toward their mother's odor to facilitate mother-infant attachment. In contrast, during the first 10 days, amygdala activation in response to threats is attenuated due to low neonatal cortisol levels, resulting in an approach response to the aversive stimuli (Moriceau & Sullivan, 2004). At postnatal day 10, stress-induced cortisol in young rats increases to adult-like levels, which in turn facilitates amygdala activation allowing fear conditioning to emerge. This plasticity in rats continues to develop into adolescence (for review see (Landers & Sullivan, 2012).

The ability of rodents to learn CS-UCS associations manifests in a way that reflects important developmental differences across sensory modalities. The ability to

learn associations may change during development, as the organism acquires new capacities to encode details of stimuli in particular sensory modalities. For example, associative learning of olfactory and gustatory CS occurs earlier than auditory and visual CS in the rodent as these modalities mature differentially with development (for review see (Richardson & Hunt, 2010)). Additionally, the expressions of learned associations may continue to change as further development supplies the maturing organism with an increasingly complex behavioral repertoire. For instance, rats as young as 16 days can express learned associations between olfactory or visual CSs and a shock-UCS, as measured by freezing behavior and heart rate; however, the presence of such associations are not expressed in measures of fear potentiated startle, which do not manifest until 23 days of age (for review see (Richardson & Hunt, 2010)). These complex processes influence the inferences that can be drawn about development and fear learning. The degree to which fear learning might appear mature or immature will depend on the particular stimuli used during learning and the behavioral modality through which learning is probed.

*Extinction*

Research on the neural mechanisms underpinning extinction learning highlight the importance of three neural structures: the amygdala, the ventromedial prefrontal cortex (vmPFC), and the hippocampus. All three structures play a major role in extinction learning with differential involvement over time and across contexts. Inhibitory circuits comprised of intercalated neurons in the amygdala, relay inhibitory outputs to the central nucleus in the amygdala preventing neuronal excitation to the same brain regions that control fear (Royer & Pare, 2002). Additionally, “infra-limbic” cortex, which lies ventral

to the prelimbic cortex in the rodent, appears to attenuate the expression of fear responses through connections with these so-called intercalated inhibitory cells within the amygdala (Quirk & Mueller, 2008). Lastly, findings also suggest that the hippocampus plays a role in mediating context-specific retrieval of fear extinction (Corcoran & Maren, 2001, 2004).

The amygdala plays a role in fear extinction processes across development. In adult rats undergoing extinction, the amygdala supports forming of the initial CS-no-UCS association. However, once this association is formed, the amygdala is no longer needed for subsequent extinction processes (Laurent, Marchand, & Westbrook, 2008). Similar findings occur in 24-day-old rats, but unlike at older ages, these re-extinction processes continue to be dependent on the amygdala in younger (i.e., 17-day-old rats) (Kim & Richardson, 2008). Thus, development results in a shift from amygdala-dependent to amygdala-independent extinction.

Likewise, developmental findings emerge for the vmPFC. In adult rats, vmPFC damage impacts extinction retrieval 24 hours after extinction but not within-session extinction (Lebron, Milad, & Quirk, 2004; Quirk, Russo, Barron, & Lebron, 2000). These results emphasize the difference between acquisition of extinction and its subsequent retrieval (i.e. extinction recall) in both the neural and the behavioral levels. Similar results were found for 23-day-old (preadolescent) and 35-day-old (adolescent) rats, with greater impairment in extinction retention in adolescent rats compare to their younger and older counterparts (Kim, Li, & Richardson, 2011; McCallum, Kim, & Richardson, 2010). Unlike PFC involvement during extinction which shows a linear relationship across developmental stages, these findings may reflect a non-linear developmental trajectory of

the PFC function during extinction retention. Some controversy exists concerning the presence of such non-linearity, which may also manifest in changes in PFC volume during adolescence, in both rats and humans (Casey & Durston, 2006; Siobhan S. Pattwell, Casey, & Lee, 2013; Shaw et al., 2008). Other work more consistently finds linear changes in brain volume and behavior during adolescence, without clear evidence of non-linear discontinuities (Steinberg, 2005). Regardless, development influences extinction within the infralimic cortex.

Finally, developmental differences also emerge for the hippocampus (Corcoran, Desmond, Frey, & Maren, 2005). During fear conditioning and extinction, spatial aspects of the surroundings are also integrated in the learning processes (Maren, 2011). Although evidence of long-term contextual memories emerges in preadolescent rats between 18 and 23 days after birth (Rudy & Morledge, 1994), only 24-day-old and not 17-day-old rats show renewal and reinstatement effects (Gogolla, Caroni, Luthi, & Herry, 2009; Kim & Richardson, 2007a, 2007b; Storsve & Richardson, 2009). These findings suggest that during early stages of development, the hippocampus could mediate within-session extinction even before it reaches full maturation, but is not involved in retrieving extinction memory at later assessments (Corcoran, et al., 2005; Delamater, 2004; Kim & Richardson, 2010).

Taken together, the available developmental data from research in animal models suggest an essential difference in the neural architecture underlying fear extinction across development. More specifically, fear extinction during early development may depend primarily on the amygdala, whereas joint roles for the amygdala, vmPFC and the hippocampus may occur at later ages. These findings may reflect neural processes that

are undergoing maturation (amygdala and hippocampus) as well as structural changes (PFC) across the developing rodent.

Developmental differences in rat models have also emerged from pharmacological studies. Studies using adult rats examining the formation of long-term extinction memory have implicated N-methyl-D-asparate (NMDA) involvement in fear conditioning and extinction (Lattal, Radulovic, & Lukowiak, 2006; Miserendino, Sananes, Melia, & Davis, 1990). Interestingly, a strong NMDA antagonist (MK-801) impairs long-term extinction in pre-adolescent but not in younger rats (Langton, Kim, Nicholas, & Richardson, 2007). Moreover, inhibitory mechanisms involved in fear extinction have been associated with increased γ-aminobutyric acid (GABA) binding in the amygdala (Chhatwal, Myers, Ressler, & Davis, 2005). Similar to NMDA results, GABA antagonist (FG7142) has been shown to attenuate extinction in adult rats (Harris & Westbrook, 1998), and in pre-adolescent rats but not in younger rats (Kim & Richardson, 2007b).

***Human Studies***

Functional magnetic resonance imaging (fMRI) studies have used classical fear conditioning paradigms to examine fear responses to discrete CSs (LaBar, Gatenby, Gore, LeDoux, & Phelps, 1998), extinction learning (Milad et al., 2007; Phelps, Delgado, Nearing, & LeDoux, 2004), and context conditioning (Alvarez, Biggs, Chen, Pine, & Grillon, 2008; Lang et al., 2009). In addition, data from lesions studies on patients complement these data from imaging studies (LaBar, LeDoux, Spencer, & Phelps, 1995; Weike et al., 2005). Consistent with animal models, this literature highlights the

importance of the amygdala, vmPFC, and hippocampus as the primary brain regions involved in fear and extinction learning in humans.

*Fear conditioning*

Similar to findings in animal models, the amygdala has also been implicated in fear learning in humans (Costafreda, Brammer, David, & Fu, 2008; Delgado, Olsson, & Phelps, 2006; Sehlmeyer et al., 2009; Sergerie, Chochol, & Armony, 2008). fMRI studies examining amygdala activation during fear conditioning paradigms have found increased amygdala activation to CS+ during fear acquisition (Buchel, Dolan, Armony, & Friston, 1999; LaBar, et al., 1998; Sehlmeyer, et al., 2009). Lesion studies in humans support the central role of the amygdala in learning about safety and danger cues. This line of research could potentially elucidate the casual contribution of the amygdala and other relevant brain structures by determining if damage to these brain areas affects fear learning and extinction. Studies conducted on patients with amygdala lesions report impairments in fear conditioning (LaBar, et al., 1995; Weike, et al., 2005). For instance, amnestic patients with damage to the hippocampus, but intact amygdala, show increased skin conductance response (SCR) during fear condition paradigms despite their inability to explicitly report the CS-UCS contingency (Fried, MacDonald, & Wilson, 1997). In contrast, patients with damage to the amygdala demonstrate awareness to the CS+-UCS contingencies but fail to show elevated physiological arousal when presented only with the CS+ (Phelps, 2006). Finally, a study in war veterans found that damaged amygdala was associated with reduced levels of fear symptoms manifested in post-traumatic stress disorders (Koenigs et al., 2008). Taken together, brain imaging and lesion studies in

humans converge with data from animal models demonstrating the conserved functionality of the amygdala in fear conditioning across species.

Some have suggested that early in life, the amygdala plays an even stronger role in fear learning (LaBar, et al., 1998). For example, human amygdala lesions early in life more strongly impair the processing of fearful facial expression (Adolphs, Tranel, Damasio, & Damasio, 1994; Shaw et al., 2005), than similar lesions occurring later in life (Hamann & Adolphs, 1999; Shaw, et al., 2005). These findings may allude to the role of the amygdala in learning during development, a role that diminishes once these associations have been created (Tottenham, Hare, & Casey, 2009).

To date, only one fMRI study has been published examining adolescent fear circuitry during fear conditioning ((Lau et al., 2011); see Table 1). This study found that adolescents were more likely than adults to recruit early-maturing subcortical regions (i.e., amygdala and hippocampus) during threat/safety discrimination learning. In addition, only adults' but not adolescents' engagement of late-maturing prefrontal cortex regions (i.e., dorsolateral prefrontal cortex) correlated positively with fear ratings during threat/safety discrimination learning. These findings imply that differences in the development of subcortical and prefrontal regions may account for age-related differences in threat/safety discrimination.

A recent review of fMRI and PET imaging studies on human fear conditioning suggests that in addition to amygdala, the insula and the anterior cingulate (ACC) are implicated in fear conditioning independent of the specific fear conditioning paradigm used (Sehlmeyer, et al., 2009). Other brain regions that have been implicated in fear conditioning include the hippocampus, cerebellum, thalamus, striatum, and sensory

cortices. Heterogeneity in neuroimaging results across studies is not surprising given the vast methodological differences in condition paradigms, contingency rate, the type of CS and UCS used, and the outcome measures indicating successful fear conditioning (For review see Sehlmeyer et al., 2009).

*Extinction*

Similar to findings in animal models, neuroimaging studies in humans implicate the pivotal role of amygdala, PFC and hippocampus in extinction learning. Heterogeneity in brain activation across fear extinction studies exists, with some reporting activation in ACC and insula (for review see (Milad & Quirk, 2012; Sehlmeyer, et al., 2009)).

In addition to the amygdala's role in fear conditioning, several studies examining extinction have demonstrated increased amygdala activation to CS-no UCS association (Knight, Smith, Cheng, Stein, & Helmstetter, 2004; LaBar, et al., 1998; Milad, et al., 2007). For instance, successful fear extinction has been found to be correlated with increased amygdala activation (Phelps, et al., 2004). Additionally, other data suggest that specific activation of the lateral amygdala and the orbitofrontal cortex during extinction may be subserved by the modulation of the amygdala-orbitofrontal circuitry in the expression of fear responses (Gottfried & Dolan, 2004).

Parallel to findings in the animal literature, a large body of human studies has implicated the role of the medial prefrontal cortex (mPFC) including the anterior cingulate cortex (ACC) in extinction learning (Gottfried & Dolan, 2004; Phelps, et al., 2004). Similarly, findings from neuroimaging studies have shown a significant increase in vmPFC activation during extinction recall (Kalisch et al., 2006), as well as a positive correlation between signal change in vmPFC activation and degree of extinction retention

(Milad, et al., 2007). Further evidence from structural imaging indicates that vmPFC thickness is correlated with extinction recall (Hartley, Fischl, & Phelps, 2011; Milad et al., 2005). These data allude to the similar function of the human vmPFC and the rodent infra-limbic cortex in fear extinction (Milad & Quirk, 2012).

Finally, these studies have also implicated the role of the hippocampus in contextual extinction learning in humans (Kalisch, et al., 2006; Milad, et al., 2007). More recently, connectivity between dorsal anterior cingulate cortex (dACC), left posterior hippocampus, and right amygdala was exhibited during extinction (Lang, et al., 2009). As a result, the interaction between the mPFC and the hippocampus may reflect context-specificity of extinction learning.

**Fear Conditioning and Extinction Studies in Children and Adolescents**

Fear conditioning can be thought of as a basic process that adds and magnifies what some would identify as unlearned or normative fears that occur across early childhood (fear of separation, fear of novelty). And indeed, from early on, children learn to identify potential threats, as well as safety cues, in the environment. Expressions of fear in humans to environmental stimuli follow a predictable developmental pattern in which certain fears increase and subside throughout development (for review see (Field & Davey, 2001). The replicable pattern expressed across cultures and even in some non-human primates suggests that these developmental expressions reflect significant common aspects of perception of novelty and familiarity and of corresponding motivations to approach or withdraw across species. During infancy, children tend to display greater fear toward stimuli found in their immediate environment, such as separation from a caregiver or loud noises. As children mature, anticipatory events and

abstract stimuli are integrated into fear learning behaviors. For example, children's self-report of normative fears have been found to range from small animals, physical injuries, medical fears, environmental events (e.g., thunder storms) to more supernatural phenomena (Gullone, 2000; Muris, Merckelbach, Meesters, & Van Lier, 1997; ten Berge, Veerkamp, Hoogstraten, & Prins, 2002), whereas in adolescence, individuals report greater fears associated to social contexts such as fear of failure and social rejection (Gullone & King, 1997). (Lau, et al., 2011)

Fear learning can be an adaptive and beneficial form of associative learning that aids in signaling the presence of a danger. However, this form of learning can become a source of pathology when fear becomes pervasive and interferes with normal functioning. In particular, perturbations in fear learning can occur when fear conditioned responses are triggered in the absence of the CS-UCS contingency (Lissek et al., 2005). In recent years research on information processing in anxiety shifted its focus from fear learning to fear extinction processes. Specifically, pathological anxiety involves deficient capacity to recognize safe cues, particularly ones that closely resemble threat cues (Lissek, 2012; Lissek, et al., 2005).

One of the first documented classical conditioning studies in infants was conducted by Watson and Rayner (1920). In their early studies, they demonstrated that fear can be learned through conditioning presented through repeated pairings of varied neutral stimuli (e.g., white rat, rabbit, or dog) with a loud noise (Watson & Rayner, 1920). In another study, 12 full-term infants as young as 3 months of age showed greater response magnitude to the CS+ compared to the CS- as indexed by skin conductance response (SCR) (Ingram & Fitzgerald, 1974). Results from these studies were among the

first to demonstrate the effects of simple fear learning in infants at early stages of development. Following these early studies on fear conditioning in children, research in this field have been hindered by ethical considerations in regard to the aversive nature of the UCS required to produce fear responses in developmental populations. While in the past decade there has been a rekindling of interest in research examining the emergence of fear learning in children, there are still relatively few developmental fear conditioning studies due to both ethical considerations as well as methodological limitations in finding an age appropriate and potent UCS. Studies examining fear learning differences across ages have sought to determine the developmental patterns associated to fear acquisition in children. A summary of sixteen fear conditioning studies conducted in normative and anxious samples of children and adolescents using a discrimination fear conditioning paradigm is presented in Table 1. The first part of the table reviews studies with healthy youth whereas the second part includes studies comparing healthy and anxious children and adolescents.

*Fear Conditioning*

Few studies have examined developmental differences of fear conditioning in children and adolescents. Across all studies (see Table 1), pediatric samples (healthy and anxious) show differential fear learning to the CS+ relative to the CS- (i.e., discrimination paradigm) as indexed by psychophysiological measures and/or subjective ratings. Results from these studies show that fear acquisition is present in typically developing children as early as 2 years of age (Ingram & Fitzgerald, 1974) with older children showing increased CR (e.g., CS+ > CS-) compared to younger children (Gao et al., 2010; Glenn et al., 2012). More specifically, children between the ages of 5 and 6 years show

36% greater stimulus discrimination compared to younger children (2-to-4 year olds)(Block, Sersen, & Wortis, 1970), as well as differences in SCR, particularly in response to the onset and absence of CS+, and temporal expectancies to the CS+(Gao, Raine, Venables, Dawson, & Mednick, 2010). Overall, these studies document that children are capable of relatively simple forms of fear learning at early stages of childhood with subsequent age differences emerging as more complex forms of fear learning continue to develop into adulthood.

Differences in fear learning and associated behaviors may be a function of changes in the brain networks subserving fear conditioning that occur across development (Lau, et al., 2011).

In one study, adolescents (10-to-17 year olds) subjectively reported less differential fear, suggesting reduced discrimination between the CS+ and the CS- compared to adults (18-to-50 year olds)(Lau, et al., 2011). In addition, imaging data allude to neural differences underlying the recruitment of brain regions in adolescents and adults. Lau et al. (2011) propose that subcortical regions (e.g., amygdala and hippocampus) play a large role in fear learning in adolescents; however with cortical maturity, adults showed greater recruitment of regions in the prefrontal cortex (e.g., dlPFC) during differential learning.

The six studies comparing anxious and non-anxious children have yielded mixed findings regarding differences in fear conditioning. Results based on subjective ratings showed that anxious children rated the CS+ as more unpleasant than the CS-; whereas, non-anxious children did not report differences in CS ratings during fear acquisition (Craske et al., 2008; Waters, Henry, & Neumann, 2009). However, in a different study, both anxious and non-anxious children showed differential learning to the CS+ and CS-,

although anxious children reported greater overall fear ratings to CS+ (Britton et al., 2013; Lau et al., 2008). Yet in another study, anxious children failed to report differential learning to the CS-UCS contingency (i.e., no differences between CS+ and CS-) while non-anxious children reported expected learning effects (Liberman, Lipp, Spence, & March, 2006).

The aforementioned studies demonstrate that children are generally capable of fear conditioning from an early age. Nevertheless, it is still unclear how these processes may be related to the vast neural changes associated with this period of development and whether differences in fear learning would emerge by using longitudinal designs.

*Extinction*

Numerous studies using self-report of normative fears indicate that fearfulness generally subsides with age (Field & Davey, 2001). One possibility is that as children mature they are better able to extinguish previously learned associations thus resulting in more effective regulation of their emotions. As a result, developmental differences in the ability to extinguish fear may be more pronounced throughout childhood and adolescence (S. S. Pattwell et al., 2012). There are, however, few studies examining the differences in extinction of learned fear behaviors in typically developing children. Four studies that examined fear extinction found expected patterns of extinction in paradigms that utilized geometric shapes (CS) and aversive tones (UCS) in both SCR and self-reported ratings (Neumann, Waters, & Westbury, 2008; Neumann, Waters, Westbury, & Henry, 2008). Extinction learning was less strong in paradigms that have used social stimuli such as affective faces (Haddad, Lissek, Pine, & Lau, 2011). In one study, adolescents were presented with gender and age matched photographs of neutral expressions (CS) that

were followed by three socially-valenced UCS (e.g., happy face with auditory "you are nice", angry face with auditory "I don't like you", and neutral face with auditory "I live in Bristol") (Haddad, et al., 2011). Extinction results showed less self-reported fear to negative CS+ relative to the neutral and positive CS+, although results did not return to pre-acquisition baseline levels. As evidenced in these findings, poor extinction of CR may be associated with prior experiences with the stimuli (Britton, et al., 2013; Britton, Lissek, Grillon, Norcross, & Pine, 2011; Pine, Helfinstein, Bar-Haim, Nelson, & Fox, 2009).

## Methodological and Ethical Considerations for Conducting Fear Conditioning Studies in Youth

In the following section, we will address methodological and ethical issues from the aforementioned studies conducted in children and adolescents. Specifically, we will review the most commonly used UCS, ITI, and CR.

### *Unconditional Stimuli (UCS)*

Successful fear conditioning and fear extinction in humans and rodents is highly dependent on the selection of a strong, potent, and biologically relevant UCS, usually electric shock (Britton, et al., 2011; Neumann & Waters, 2006). However, electrical shock presents the risk of causing pain or increased levels of anxiety and generally cannot be used with child populations (Neumann, Waters, & Westbury, 2008; Neumann, Waters, Westbury, et al., 2008; Pine, et al., 2009). As a result, one of the major limitations in examining fear learning in children is the selection of a developmentally appropriate UCS while still preserving its potency and novelty.

As described in Table 1, 6 out of the 16 studies used ecologically valid UCS such as loud car horns (Block, et al., 1970); loud sounds of metal jangling objects (Gao, et al., 2010), aversive noises (e.g. metal scraping on slate) (Neumann, Waters, & Westbury, 2008; Neumann, Waters, Westbury, et al., 2008), and negatively-associated comments (Haddad, et al., 2011). The use of one specific ecologically valid UCS (e.g., a 83 dB sound of a three-pronged garden tool being scraped across slate) yielded reliable fear acquisition and extinction effects across 8-to-11 year old children (Neumann, Waters, Westbury, et al., 2008), 13-to-17 year old adolescents (Neumann, Waters, & Westbury, 2008), and adults (Neumann & Waters, 2006). Five studies used stimuli such as pure tones (1000 Hz) or tones combined with white noise (105-110 dB), in various duration ranging from 200 ms to 4000 ms (Craske, et al., 2008; Liberman, et al., 2006; Pliszka, Hatch, Borcherding, & Rogeness, 1993; Waters, et al., 2009). One potent fear conditioning paradigm that was tested successfully with children and adolescents used social stimuli (images of human faces) paired with an aversive scream (Britton, et al., 2013; Glenn et al., 2012; Lau, et al., 2011; Lau, et al., 2008). This UCS was found to be comparable to an alarm, a loud tone, and white noise as measured by subjective self-report (Britton, et al., 2011). Further, a recent study found that although subjects reported shock to be more aversive than the "screaming lady", both paradigms yielded similar differential conditioning effects as evidenced by larger FPS magnitudes to the CS+ relative to the CS- (Glenn, Lieberman, & Hajcak, 2012).

Given the variability in the selection of UCS across studies, it may be possible that these methodological differences produced inconsistencies in CR magnitudes rather than capture developmental processes in fear learning. For example, it is unclear whether

disparities among adolescents and adults in their resistance to extinction (Pratwell et al., 2012) result from cortical maturation or, if these differences stem from variability in the aversive properties of the UCS (e.g., loud sounds vs. shock) (Pine et al., 2001). The UCS potency (e.g., weak vs. strong UCS) may also explain variability in fear conditioning studies (Britton et al., 2010). While some studies use loud sounds as the UCS, the potency of the stimulus (i.e., sound pressure levels) varies across paradigms. For instance, two separate studies used a 1000Hz pure tone as the UCS but in one study it was administered at 107dB for 1s (Craske et al., 2008) while in another study it was presented at 105dB for 500ms (Liberman, Lipp, Spence, & March, 2006). Another example of this issue can be exemplified in studies using the fearful face and scream as the UCS where audio stimuli have been presented at 80dB (Glenn et al., 2012), 90dB (Lau et al., 2011), and 95 dB (Lau et al., 2008; Britton et al., in press). Therefore, the aversive properties of the UCS can ultimately impact the magnitude of the CR and the degree of fear learning across paradigms.

*Inter-trial Intervals (ITI)*

Another methodological issue concerns the duration of inter-trial intervals (ITI) selected for fear conditioning paradigms in pediatric populations. The selection of the ITI is crucial in maximizing fear conditioning effects as it allows both physiological reactivity and behavioral responses to return to baseline activity. Given that ITI selection is a function of the task paradigm coupled with methodological requirements of the dependent variable, researchers are presented with the challenge of findings a balance between task reliability and participant compliance. Some data from animal and human studies suggest enhanced conditioning effects when selecting longer ITI periods (Barela,

1999; Prokasy & Ebel, 1964). The implementation of long ITI periods extend the total duration of the task, resulting in increased boredom, restlessness, and fatigue effects. These effects are particularly relevant to studies in children. The duration of ITI in child fear conditioning paradigms have commonly varied from 13-16s to 20-30s (see Table 1). To date, there have not been any empirical studies that systematically examined the effects of ITI durations on fear conditioning in child studies. This issue is further complicated with fear conditioning paradigms used in the context of neuroimaging studies where some studies typically use ITIs less than 10s (Lau, et al., 2011) while others use ITIs ranging between 12-18s (Milad, et al., 2007). Specifically, the use of longer ITIs in imaging studies may be impractical given the tradeoff between task duration and number of trial presentations in a task, as well as an increase in the probability of movement artifacts.

*Conditioned Response (CR)*

Various methodologies have been used to measure CR in fear conditioning paradigms. In human studies, fear acquisition is often indexed using implicit measures of physiological arousal, such as skin conductance response (SCR) and fear potentiated startle (FPS). Additionally, most studies with human subjects use explicit self-reported measures of fear and anxiety levels. As shown in Table 1, SCR ($n$ = 11) and self-report ($n$ = 11) are used with similar frequency to measure CR, where the majority of studies use more than one dependent variable. Studies in children show that both SCR and self-report are reliable measures of fear learning during fear acquisition (Britton, et al., 2013; Gao, et al., 2010; Lau, et al., 2011; Morrow, Boring, Keough, & Haesly, 1969; Neumann, Waters, Westbury, et al., 2008).

Some studies have found inconsistencies between physiological results and subjective verbal ratings, primarily within anxious pediatric samples. For instance, in one study, anxious and non-anxious children did not differ in verbal self-report of arousal although differences in SCR were observed (Craske, et al., 2008). Another study reported discrepancies during initial phases of extinction between self-reported measures of UCS expectancies and physiological measures of SCR and FPS (Neumann, Waters, Westbury, et al., 2008). Findings have also revealed that anxious children show resistance to extinction, as measured by SCR, and no differences on self-reported measures of arousal relative to controls (Waters, et al., 2009). Finally, Britton et al., (2013) found anxious adolescents report more fear to the CS+ and CS- during conditioning and extinction phases while no differences emerged in SCR or FPS when compared to non-anxious adolescents.

Although self-reported measures are common in the adult literature, the use of self-report in younger children raises concerns regarding whether children are able to provide reliable explicit judgments concerning CS-UCS contingencies. Thus, the addition of physiological measures may provide converging information regarding differences among autonomic and subjective measures of learning. Nevertheless, it is important to highlight that the overall physiological measures may also reflect changes in both physiology and brain regions as a function of age. For instance, one possible account for SCR differences in fear conditioning may result from sweat physiology across various developmental periods (e.g., childhood vs. puberty); however, difference between CS+ and CS- should not be affected.

To date, very few developmental studies have reported using FPS (*n*= 4), EKG (*n*= 2), or fMRI (*n*= 2) in conjunction with either SCR or self-report. In particular, the use of FPS as a measure of CR is advantageous in that it is able to capture cross-species (e.g., human and non-human animals) physiological responses to valence-specific states (Grillon, Ameli, Woods, Merikangas, & Davis, 1991). While the inclusion of multiple psychophysiological measures may hinder the feasibility of fear conditioning studies in children, findings from these studies can provide converging evidence on the interplay between different autonomic measures of fear learning. In summary, SCR and self-report are the most widely used measures in fear conditioning studies with children. However, more studies are needed to determine how these measures are related to the developmental processes involved in the emergence of fear learning in children.

## Conclusion and insights for future developmental research on fear conditioning

There is a need for research on fear conditioning processes in children in order to understand the underlying behavioral, physiological, and neural mechanisms associated with normative and pathological fear learning across development. The majority of studies conducted on this topic have primarily focused on fear acquisition and extinction during late childhood and adolescence but rarely have they focused on infancy or early childhood. Given the developmental changes children undergo throughout these crucial periods, behavioral and neurological differences in fear learning are expected to emerge as a function of age. A translational developmental neuroscience approach is particularly advantageous given the strong behavioral and neurological conservation of underlying fear circuitries and learning processes across human and nonhuman species. The use of

well-controlled fear conditioning paradigms may offer insights into these developmental trajectories by enabling a systematic examination of basic fear related mechanisms and associative learning processes. Given the suggestions provided in this review, we hope to have mitigated some of the ethical and methodological difficulties that have hindered this line of research.

Research in animal models conducted in parallel with human studies has identified developmental differences among cortical and subcortical brain regions at certain ages. In particular, findings from these studies indicate that the neural circuitries underpinning fear learning and fear extinction are mediated by different brain regions which are subsequently evidenced by differences in behavioral outcomes. In addition, changes in functional connectivity among different brain regions are also expected to evolve with age and thereby to affect behavior (Gee et al., 2013; Guyer et al., 2008; Kim, Hamlin, & Richardson, 2009). And indeed, fear conditioning emerges early in development but extinction, in particular the ability to retrieve extinction memory, emerges later in development. These results highlight the need for further translational work that will examine the emergence of these learning processes in human children, adolescents, and adults via cross-sectional and longitudinal designs.

In addition to focusing on typical development, this line of research impacts the understanding the etiology and treatment of pediatric anxiety. Anxiety disorders are among the most prevalent forms of childhood psychopathology (Verhulst, van der Ende, Ferdinand, & Kasius, 1997). While some anxiety disorders are transient throughout development, recent studies suggest that pediatric anxiety disorders commonly persist into adulthood (Bruce et al., 2005; Hasler et al., 2005; Perkonigg et al., 2005). Because

anxiety disorders are costly and debilitating conditions that are very often associated with other severe psychopathology (Achenbach, 1995; Pine, Cohen, Gurley, Brook, & Ma, 1998), there is an imperative need to identify early risk and resilience factors that moderate pediatric anxiety to chronic illness. Research examining fear extinction in pediatric populations has great relevance for understanding of learning processes that facilitate effective exposure therapy for anxiety disorders. For example, exposure therapy which is one of the most effective treatment for pediatric and adults anxiety disorders relies heavily on extinction learning processes mediated by the vmPFC. Based on the available data reviewed in the paper, future research should focus primarily on differences in extinction, extinction retention, and children's ability to differentiate between threatening and non-threatening stimuli (i.e., danger vs. safety) as potential targets for prevention and treatment strategies. The increasing learning capabilities along with the brain plasticity that occur throughout development provide a unique opportunity to alter anxiety trajectories and prevent long-term psychiatric morbidity.

Table 1. Summary of developmental fear conditioning studies in healthy and anxious youth. The first part of the table reviews studies on healthy youth followed by studies examining anxious youth

| Authors | Dx | N | Age (years) | CS | UCS | DV | Pre-exposure | | # of Trials | | | Main Results |
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| | | | | | | | CS | UCS | ACQ | EXT | ER | |
| **HEALTHY YOUTH** | | | | | | | | | | | | |
| Block, Sersen, & Wortis (1970) | Hv | 77 | 2-11 | 400 Hz tone or 1000 Hz tone for 5s (55dB) | Loud sound for 1s: auto horn (95dB) | EKG | 0 | 0 | CS+:10 CS-:10 | NA | NA | Two-to-4 year olds failed to display cardiac discrimination (i.e., lack of conditioning).<br>Four-to-6 year olds showed partial evidence for conditioning.<br>Six-to-11 year olds showed evidence for conditioning. |
| Gao, Raine, Venables, Dawson, & Mednick (2010) | Hv | 200 | 3,4,5, 6,8 | 1000 Hz or 500 Hz for 12.5s (60 dB) | Loud sound for 4.5s: white noise in tin can with metal jangling objects (95 dB) | SCR | Neutral tones:6 | 0 | CS+:9 CS-:3 | NA | NA | Children as young as 3years old showed fear conditioning (CS+>CS-).<br>Differential SCR responses increased with age, particularly in 5-to-6 year olds. |

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| Glenn, Klein, Lissek, Britton, Pine, & Hajcak (2012) | Hv | 40 | 8-13 | 2 neutral female faces for 6s | Fearful face (3s) + scream for 1s (80dB) | FPS S.R. | CS+:4 CS-:4 | 0 | CS+:8 CS-:8 | 0 | CS+:8 CS-:8 GS:8 | Self-report ratings revealed marginally significant differences from pre- to post-task (increase in CS+ and decrease in CS-).<br>During acquisition, FPS results revealed larger startle magnitudes (CS+>CS-).<br>Older children were better able at discriminating threat from safety cues. |
| Haddad, Lissek, Pine, & Lau (2011) | Hv | 42 | 12-15 | 3 neutral faces for 3s | CSnegative: angry face + criticism CSpositive: happy face + compliment CSneutral: neutral face + neutral commen | S.R. | CSnegative:2 CSpositive:2 CSneutral:2 | 0 | CS+:9 CS-:9 CS-:9 | CS+:8 CS-:8 CS-:8 | NA | Adolescents reported greater subjective fear to the CSnegative compared to CSpositive and CSneutral post-acquisition.<br>After extinction, differences across CSs persisted with subjects reporting less pleasantness to CSnegative compared to CSpositive and CSneutral. |

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| Lau et al. (2011) | Hv | Total:42 21 adolescents and 21 adults | *M* adolescents= 13.09 *M* adults = 27.10 | 2 neutral female faces for 5s | Fearful face for 3s + scream for 1s (90dB) | SCR S.R. | CS+:8 CS-:8 | 0 | CS+:10 CS-:10 | NA | NA | During fear acquisition, all subjects showed greater SCR to the CS+ than CS-; adolescents showed greater overall SCR than adults.<br>All subjects reported greater subjective fear to the CS+ than CS-. |
| | Hv | Total:35 15 adolescents and 20 adults | 10-17; 18-50 | 2 neutral female faces for 6s | Fearful face + scream for 1.1s (90dB) | S.R. fMRI | CS+:3 CS-:3 | 0 | CS+:60 CS-:60 | NA | NA | Both groups showed differential learning (CS+>CS-), but adolescents reported greater fear to the CS+ and less discrimination between CSs compared to adults.<br>Adolescents showed increased amygdala and hippocampal activation to the CS+ versus CS- trials than adults.<br>Age differences were also found in subjective fear ratings of the CS- and dorsolateral prefrontal cortex |

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| | | | | | | | | | | | | (dlPFC) activation. |

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| Morrow, Boring, & Keough (1969) | Hv | 42 | 10-12; 19-21; 62-75 | 3 horizontally or 3 vertically arranged white lights for 5.1s | Electric shock for 100 ms | SCR | CS+:4 CS-:4 | 3 | CS+:20 CS-:20 | NA | NA | Children and young adults showed increased SCR to the CS+ and overall decline to the CS- during conditioning. No learning differences were found between the age groups. |
| Neumann, Waters, & Westbury (2008) | Hv | 15 | 13-17 | 2 geometric shapes for 8s: diamond or triangle | Loud sound for 3s: three-pronged garden fork scrapping slate (83 dB) | FPS SCR S.R. | 0 | 0 | CS+:8 CS-:8 | CS+:8 CS-:8 | NA | Adolescents showed fear conditioning (CS+>CS-) and extinction across all three DVs. |
| Neumann, Waters, Westbury, & Henry (2008) | Hv | 16 | 8-11 | Two geometric shapes for 8s: white 10-cm square or black 30-cm square | Loud sound for 3s: metal scrapping slate (83 dB) | SCR S.R. | CS+:2 CS-:2 | 0 | CS+:12 CS-:12 | CS+:12 CS-:12 | NA | Eight-to-11 year olds showed fear acquisition (CS+>CS-) and extinction as evidenced by SCR and self-report of arousal and unpleasantness. |

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| Pattwell et al. (2012) | Hv | 83 | 5-11; 12-17; 18-28 | 2 colored squares: blue or yellow presented for 3s | White noise combined with 1000Hz (85-107dB) for 1s | SCR | 0 | 0 | CS+:24 CS-:24 | CS+:24 CS-:24 | NA | Participants across all age groups showed differential fear learning (CS+>CS-) during fear acquisition. Age differences emerged during extinction with adolescents showing dampened extinction compared to children and adults. |
| **ANXIOUS YOUTH** | | | | | | | | | | | | |
| Britton et al. (2013) | Hv, GAD, SOC, SAD | Total:65 Hv:42 ANX:23 | 8-19 | 2 neutral female faces for 7-8s | Fearful face + Scream for 1s (95dB) | FPS SCR S.R. fMRI | CS+:4 CS-:4 | 0 | CS+:10 CS-:10 | CS+:8 CS-:8 | *M*=20 days CS+:1 CS-:1 GS:9 | During acquisition and extinction, relative to healthy youth, anxious youth reported greater fear to both CSs.<br>All groups showed similar fear conditioning (CS+>CS-) and extinction across all three DVs.<br>During extinction recall, anxious adolescents exhibited lower sub-genual anterior cingulate (sgACC) activation |

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| | | | | | | | | | | | | compared to their healthy peers when appraising threat. In ventromedial prefrontal cortex (vmPFC), anxious adolescents showed greater activation to the most extreme CS+ and CS- displaying a U-shaped pattern of activation. |
| Craske et al. (2008) | Hv, SAD, PD, GAD, SOC | Total:49 Hv:11 ANX:23 At risk:15 | 7-12 | 4 geometric shapes for 8s: trapezoid (CS+), triangle (CS-), circle, rectangle | 1000Hz pure tone for 1s (107 dB) | SCR S.R. | CS+:1 CS-:1 Circle:1 Triangle : 1 | 0 | CS+:8 CS-:8 | CS+:4 CS-:4 | *M*=12 days CS+:4 CS-:4 | All groups showed conditioning effects (CS+>CS-) reflected in SCR. During extinction and extinction recall, anxious children showed elevated levels of SCR to CS+ and CS- compared with non-anxious children. Anxious children showed resistance to within-session extinction and extinction retest at 2-week follow-up. |

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| Lau et al. (2008) | Hv GAD, SOC, SAD | Total:54 Hv:38 ANX:16 | *M*= 13.64 | 2 neutral female faces for 8s | Fearful face + scream for 3s (95 dB) | S.R. | CS+:4 CS-:4 | 0 | CS+:16 CS-:16 | CS+:3 CS-:3 | *M*=16 days CS+:12 CS-:12 | During acquisition, all subjects rated the CS+ as more fearful than CS-, however anxious adolescents' ratings to the CS+ were greater compared to healthy subjects.<br>During extinction, both groups rated the CS+ as more aversive compare to the CS-, indicating stability even post- extinction. |
| Liberman, Lipp, Spence, & March (2006) | Hv, SOC, GAD, SAD, SP | Total:83 Hv:30 ANX:53 | 7-14 | 4 neutral cartoons for 5s: 4 cartoons during pre-exposure and 2 cartoons during acquisition (CS+=1; CS-=1 ) | 1000Hz Pure tone for 500 ms (105 dB) | FPS SCR S.R. | CS+: 1 CS-: 1 Cartoons:2 | 0 | CS+:6 CS-:6 | CS+:8 CS-:8 | NA | During acquisition, healthy children rated the CS+ as more arousing than CS-, but no differences were found in anxious children.<br>After extinction, healthy children showed no differences in fear ratings between CS+ and CS- whereas anxious children rated the CS+ as more fearful than the CS-.<br>During acquisition, |

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| | | | | | | | | | | | | SCR and FPS did not reveal conditioning effects in both groups.<br>However, anxious children showed increased FPS during extinction to the CS+ compare to the CS-, whereas healthy children showed no difference. |
| Pliszka, Hatch, Borcherding & Rogeness (1993) | Hv, ANX, ADHD | Total:56<br>Hv:22<br>ANX:11<br>ADHD:23 | 6-12 | 2 geometric shapes + tone for 4s: red square + 1000 Hz tone or blue square + 500Hz tone | White noise for 200 ms (110 dB) | SCR<br>EKG | 0 | 0 | CS+:8<br>CS-:8 | CS+:4<br>CS-:4 | NA | During acquisition, subjects showed differential conditioning (CS+>CS-) in both SCR and EKG, with no group differences.<br>Extinction was observed only in EKG but not it SCR. |
| Waters, Henry, & Neumann (2009) | Hv, SOC, GAD, SP | Total:35<br>Hv:18<br>ANX:17 | 8-12 | 4 geometric shapes for 8s: CSs were trapezoid or triangle and two additional shapes of a circle and a rectangle | 1000Hz Pure tone for 1s (107 dB) | SCR<br>S.R. | CS+:1<br>CS-:1<br>Circle:1<br>rectangle:1 | 0 | CS+:8<br>CS-:8 | CS+:4<br>CS-:4 | NA | Compared to healthy subjects, anxious children showed increased SCR to both CSs and reported the CS+ to be more arousing compared to the CS- during acquisition.<br>At the extinction phase, anxious |

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| | | | | | | | | | | | | children showed increased SCR to the CS+ but no differences in their subjective ratings between stimuli. |

*Note*: All reported studies used a discrimination fear conditioning paradigm. Dx = disorder; Hv = healthy volunteers; ANX = anxiety disorder; ADHD = attention deficit hyperactivity disorder; SOC = social anxiety disorder; GAD = generalized anxiety disorder; SAD = separation anxiety disorder; SP = specific phobia; PD = panic disorder; N = number of subjects; CS = conditioned stimuli; UCS = unconditioned stimulus; GS = generalization stimuli; DV = dependent variable; EKG = electrocardiogram; SCR = skin conductance response; S.R. = self-report; FPS = fear potentiated startle; fMRI = functional magnetic resonance imaging; ACQ = acquisition phase; EXT = extinction phase; ER = extinction re-recall/test; NA= not applicable.

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CHAPTER 2

## Introduction

Children learn to adjust their behavioral responses to events and cues in their surroundings at an early age. This cognitive process is crucial in helping individuals identify potential threats and safety cues from the environment. In humans, fear reactions to environmental threats have been found to follow a predictable pattern with certain fears increasing and subsiding (e.g., fear of separation, fear of novelty) across development (for review see Field & Davey, 2001).Differences in fear learning and associated behaviors may result from maturational changes in brain networks underlying fear conditioning (Hare, Tottenham, Galvan, Voss, Glover, & Casey, 2008; Lau, Britton, Nelson, Angold, Ernst, Goldwin et al., 2011; Gee et al., 2013a). However, the exact developmental patterns of learning-related processes, such as fear conditioning, extinction, and generalization of acquired fears, in humans remains unclear.

Fear conditioning is the most commonly used experimental paradigm for investigating the neural mechanisms and psychophysiological processes underlying learning about threat cues in animals and humans (LeDoux, 2000). A form of associative learning, classical fear conditioning involves the presentation of a simple visual or auditory stimulus (neutral stimulus; NS) followed by the pairing of an aversive stimulus (unconditioned stimulus; US). Through repeated presentations of the pairing, a fear conditioned response (CR) is produced indicative fear learning. Extinction learning refers to when the CS is presented in the absence of the aversive US (CS-no US); resulting in a gradual weakening in the CR (Bouton, Westbrook, Corcoran, & Maren, 2006; Sotres-Bayon, Cain, & LeDoux, 2006). Studies support that the initial CS-US association remains intact and a new association (i.e., CS-no US) is formed during fear

extinction (Quirk & Mueller, 2008). To examine the acquisition of fear established through the relationship between the CS and US, many studies use discrimination conditioning paradigms which are characterized by the presentation of two CS where one stimulus (CS+) is paired with the US while a different neutral stimulus is presented in the absence of the US (CS–) (Delgado, Olsson, & Phelps, 2006; Vansteenwegen, Iberico, Vervliet, Marescau, & Hermans, 2008). Fear learning, as measured with FC paradigms, is often indexed in adults by autonomic responses, such as sweat (e.g., skin conductance response), fear potentiated startle, heart rate, and subjective fear ratings (LeDoux, 2000).

While fear learning can be an adaptive and beneficial form of learning that aids in signaling the presence of danger, this type of associative learning can become a source of pathology when CRs are triggered in the absence of the CS-UCS contingency (Davis, Falls, & Gerwirtz, 2000; see Lissek et al., 2005). Impairments in fear response inhibition to safety cues have been implicated in the etiology and treatment of anxiety disorders (Field, 2006; Mineka & Oehlberg, 2008). Anxiety disorders are among the most common psychiatric disorders with prevalence rates averaging between 10-20% of pre-adolescent children (Cartwright-Hatton, McNicol, & Doubleday, 2006) and are predictive of other forms of internalizing disorders in adolescence and adulthood (Perez-Edgar & Fox, 2005; Pine, 2007). Associative learning theories posit that deficits underlying mechanisms are related to threat and safety discriminations, a process referred to as fear generalization (Lissek, 2012). It is hypothesized that anxious individuals display elevated stimulus generalization (i.e., overgeneralize) to perceptually similar stimuli to the threat cue (CS+) which elicits a CR to a certain degree (LeDoux, 1998). By systematically varying the phenotypic similarities to the threat stimuli, an individual assesses their CR response to conditioned stimuli and generalization-stimuli (Lissek et al., 2008). Thus,

identifying developmental patterns of normative fear learning processes (e.g., fear acquisition, extinction, fear generalization) from childhood to adolescence may enhance our understanding of the etiology of anxiety disorders and inform new approaches to early detection and treatment.

Considerable research on fear learning has been conducted in adults and rodent models, however less is understood about developmental patterns associated to fear learning in healthy children from early childhood to adolescence. Watson and Rayner (1920) conducted one of the first classical conditioning studies examined in infants. In their early studies, they demonstrated that fear can be learned through conditioning presented repeated pairings of varied neutral stimuli (e.g., white rat, rabbit, or dog) with a loud noise. In another study, 12 full-term infants as young as 3 months of age showed greater response magnitude to the CS+ compared to the CS- as indexed by skin conductance response (SCR) (Ingram & Fitzgerald, 1974). Results from these studies were among the first to demonstrate the effects of simple fear learning in infants even at early stages of development. However, more complex fear learning abilities (e.g., fear extinction, generalization of fears) may continue to develop as neural changes in cortical maturation as well as structural changes emerge across childhood and adolescence (Kim & Richardson, 2010; Lau et al., 2011; Glenn, Klein, Lissek, Britton, Pine, & Hajcak, 2012).

Recent studies on fear conditioning in children have been limited due to ethical considerations regarding the aversive nature of the UCS used to elicit fear responses in child populations. Traditional fear conditioning paradigms administered in adult and animal studies involve the use of aversive stimuli, such as shock, which is unfeasible with children (Neumann, Waters, &Westbury, 2008; Pine, Helfinstein, Bar-Haim, Nelson, & Fox, 2009). As a result, the selection of an age appropriate, evolutionary relevant, and potent UCS has been a challenge to

the field as these factors influence the degree of reliable fear conditioning levels (Britton, Lissek, Grillon, Norcross, & Pine, 2011; Neumann & Waters, 2006).

To address this issue, a number of studies have used ecologically significant stimuli where children are presented with UCS such as loud car horns (Block, Sersen, & Wortis, 1970); loud sounds of metal jangling objects (Gao, Raine, Venables, Dawson, & Mednick, 2010), metal scrapping on slate (Neumann, Waters, Westbury, & Henry, 2008; Neumann, Waters, & Westbury, 2008), negatively associated objects (Field, 2000b) and social stimuli such as negative criticisms (Haddad, Lissek, Pine, & Lau, 2011) or fearful face paired with a scream (Lau et al., 2008; Lau et al., 2011; Glenn et al., 2012; Britton et al., 2013). Other studies have used non-social cues like pure tones (1000 Hz) or tones combined with white noise presented between 105-110 dB to elicit CR in children (Pliszka, Hatch, Borcherding, & Rogeness, 1993; Liberman, Lipp, Spence, & March, 2006; Craske, Waters, Bergman, Naliboff, Lipp, Negoro et al., 2008; Waters, Henry, & Neumann, 2009; Pattwell et al., 2012). One potent fear conditioning paradigm tested successfully with children and adolescents used a social stimuli (e.g., female face) paired with an aversive sound (e.g., scream) (Britton et al., 2013; Glenn et al., 2012; Lau, et al., 2011; Lau et al., 2008). This UCS was found to be comparable to an alarm, a loud tone, and white noise as measured by subjective self-report (Britton, et al., 2011).

To date, only one longitudinal study has assessed developmental trajectories of fear conditioning in children at ages 3, 4, 5, 6, and 8 years (Gao et al., 2010). Findings from this study revealed fear conditioning was present in children as young as 3 years of age with significant CR increasing from ages 5-to-6 years evidenced via SCR. Similarly, Newmann, Waters, Westbury, and Henry (2008) found robust fear conditioning (CS+ > CS-) and extinction learning in a group of typically developing 8-to-11 year olds using self-reported measures of UCS expectancy and

SCR. These findings were replicated in another study with 13- to 17-year-old adolescents as measured by fear potentiated startle and SCR (Neumann, Waters, & Westbury, 2008) and adults (Neumann & Waters, 2006).

Cross-sectional studies have found significant age differences in fear learning, extinction, and fear generalization (Lau et al., 2011; Glenn et al., 2012; Pattwell et al., 2012). In a study by Pattwell et al. (2012), researchers found successful differential fear conditioning (e.g., greater SCR to CS+ relative to CS-) among 5-to-11 year old children, 12-to-17 year old adolescents, and 18-to-28 year adults during fear acquisition. More importantly, differences among age groups emerged during fear extinction with adolescents showing dampened extinction rates when compared to children and adults. Given that adolescence is characterized by neurodevelopmental changes in prefrontal regions (Casey et al., 2010), researchers postulate that this developmental time period may reflect a qualitative different period in which extinction learning is reduced.

Using an adapted version of the Lau et al. (2008) paradigm, another study showed discriminative fear conditioning across 8-to-13 year olds children (Glenn et al., 2012).Further examination between 8-10 and 11-13 year olds revealed age differences in fear conditioning such that FPS in older children who reported contingency awareness were associated with larger startle magnitudes to the CS+ relative to younger children. Additionally, older children were better at discriminating threat from safety cues during in a fear generalization condition. Similarly, converging findings from Lau and colleagues (2011) demonstrated that adolescents show reduced discrimination to threat and safety cues in comparison to adults during a differential conditioning paradigm. Based on these few studies, findings support that children are generally capable of fear learning processes (e.g., fear conditioning and extinction), threat and safety cue discrimination improves with age, and adolescents show attenuated extinction learning

when compared to adults. Taken together, age related differences in fear learning seem to emerge as more complex forms of learning continue to mature and interact with developmental processes and changes in neural circuitry.

The main goal of the study was to examine how fear learning, extinction, and fear generalization emerge in a sample of typically developing 5-to-10 year old children. To do this, the present study used a modified version of a previously used fear conditioning paradigm (Britton et al., 2013). During the task, colored cartoon bells served as the CS and a loud alarm sound served as the UCS. These stimuli were selected based on its ecological relevance and non-facial properties (e.g., fearful face and scream) used to increase child compliance and ensure developmental appropriateness of the task paradigm. The two main goals of the study were: a) to test the feasibility of a novel fear conditioning paradigm in 5-to-10 year old children; b) to examine developmental differences in discrimination fear conditioning, extinction learning, and fear generalization among three developing age groups: 5-6 year olds, 7-8 year olds, and 9-10 year olds. First, we hypothesized that children across all age groups will show expected behavioral and physiological patterns of discrimination fear conditioning and extinction. Second, based on previous studies, we hypothesized that fear conditioning, extinction learning, and fear generalization will improve with age such that older children (9-to-10 year olds) will show more robust fear learning and generalization rates relative to younger children (e.g., 5-to-6 and 7-to-8 year olds). Skin conductance response and subjective ratings of fear to the UCS were used to index fear learning levels.

## Method

### Laboratory Visit 1

#### *Participants*

Participants were 64 typically developing children, between the ages of 5 and 10, recruited through mailings and advertisements in the Washington, DC metropolitan area. Of the children, 50 (78.1%) were Caucasian, 4 (6.3%) were African-American, 2 (3.1%) were Hispanic, 1 (1.6%) was Asian, and 7 (10.9%) were identified by their parent as "Other" ethnicities. There were 19 5-to-6 year olds (10 females; $M_{age}$ = 5.90; *SD* = .58), 17 7-to-8 year olds (10 females; $M_{age}$ = 8.10; *SD* = .57), and 18 9-to-10 year olds (10 females; $M_{age}$ = 10.07; *SD* = .51).

A total of four children discontinued participation when they became anxious or were non-compliant. Skin conductance response (SCR) data from six participants in the final sample were excluded from data analyses due to non-responders and equipment failure.

#### *Procedure*

After initial contact, 5-to-10 year old children and their primary caregiver were invited to visit the university laboratory to participate in the first assessment of the study. The current study involved data collection at two different time points. Study procedures were approved by the University of Maryland institutional review board. Upon arrival to the university laboratory, informed consent was obtained by the parent and children were briefed on the procedures and assent was obtained. For each laboratory visit, families were compensated for their time with developmentally appropriate toys totaling a value of $20 (i.e., one large toy and two small toys).

During the first laboratory visit, participants completed the pre-acquisition, fear acquisition, and extinction phase of the study. Participants' subjective responses of their current

levels of positive and negative affect to the CS+ and CS- were collected at set times throughout the first laboratory visit.

### *The Bell Conditioning Task*

The current fear conditioning paradigm is similar to that previously described by Britton et al. (*in press*). In the current study, instructions and task were slightly adapted (e.g., non-social stimuli) to increase child compliance and developmental appropriateness. Before the start of data collection, children were fitted with two Ag/AgCl electrodes that were attached to two of their fingers on the left hand and two EAR-3A earplugs. STIM Stimulus Presentation System (Version 8.464, James Long Company; Caroga Lake, NY) was used to program and present the bell conditioning task. All stimuli were presented on a desktop computer and 15" monitor. Children were seated approximately 75 cm from the computer monitor during the bell conditioning task.

Participants were informed that they could learn to predict when the UCS would occur but were not explicitly informed of the CS/UCS contingency. Throughout the task, participants passively viewed blue and yellow cartoon figures of bells selected as the conditioned stimuli (CS+, CS-). The bell selected as the CS+ was counterbalanced across participants. A differential conditioning procedure was used (Öhman, Hamm, & Hugdahl, 2000).

The Bell Conditioning task included three phases: a) pre-acquisition; b) fear acquisition; and c) extinction. The CS+ and CS- were presented for 7-8 seconds followed by a gray screen presented for 8-21seconds (*M*= 15s*).* The UCS consisted of a mildly unpleasant noise (e.g., loud alarm noise) presented at 95dB for 1 second concurrently with a red bell figure. The inter-trial interval (ITI) ranged from 11-15seconds. During the fear acquisition phase, the CS+ was followed by a UCS with an 80% reinforcement schedule (see Figure 1). The pre-acquisition and

extinction phase consisted of the CS+ and CS- presented in the absence of the UCS. SCR and subjective ratings of fear were used to index fear acquisition and extinction rates.

### *Skin Conductance Response (SCR) Recording*

SCR was recorded from two Ag/AgCl electrodes attached to the medial phalanx of the middle and the ring fingers of the left hand. SCR data recorded with an EDA bioamplifier (SA Instruments, San Diego, CA) with a gain of 10 μS per volt. The amplified signal was digitized at a sampling rate of 200 Hz and Snap-Master data acquisition software (HEM Data Corporation. Southfield, MI).

All subsequent processing and analysis of the raw SCR data was conducted using Phy General Physiology Analysis System (Version 8.464, James Long Company; Caroga Lake, NY). SCR data to the CS+ and CS- trials were quantified as the peak difference between amplitudes (within 1-7 seconds following stimulus onset) and baseline activity. SCR data were square-root transformed for each participant and were range corrected (SCR/SCRmax). Transformed data were averaged across all trials and analyzed**.** The square-root transformed SCR data to the UCS were analyzed for group effects using repeated measures ANOVA.

### *Subjective Ratings of Fear and Anxiety*

Participants completed subjective ratings of fear and anxiety levels to the CS+ and CS- using a ten-point Likert scale (1= none, 10= extreme). This form was administered prior to pre-acquisition, after fear acquisition, and after extinction. Items were comprised of questions such as “how scared are you of this blue bell?” and “how anxious were you when you saw the blue bell?”

### *Data Analysis*

To examine fear learning across phases, a repeated measures ANOVA was used to test main effects and interaction effects using age-group (5-6 year olds, 7-8 year olds, and 9-10 year olds) as between-subject factors and phase (pre-acquisition, fear acquisition, and extinction) and stimulus type (CS+, CS-) as within-subject factors for SCR data and subjective ratings. Repeated measures ANOVAs were also conducted to examine fear learning rates by dividing trials into early and late blocks for each phase (6 Early/Late Phases: early/late pre-acquisition, early/late fear acquisition, early/late extinction x 2 Stimulus Type: CS+, CS-). Lastly, learning rates were explored by analyzing two trial bins across each phase (11 Trial Bins: pre-acquisition 1-2, 3-4; fear acquisition 2, 3-4, 5-6, 7-8, 9-10; extinction: 2, 3-4, 5-6, 7-8 x 2 Stimulus Type: CS+, CS-). Follow-up *t*-tests were performed following significant interaction effects.

All statistical analyses were conducted using PASW (Version 20.0). Significant effects were detected at $\alpha = 0.05$ level. Post-hoc analyses were conducted using Bonferroni correction. Pearson correlations were used to examine relationships among age, SCR data, and subjective ratings across all participants. Significant effects were detected at $\alpha = 0.05$ level.

## Laboratory Visit 2

### *Participants*

Following the first laboratory visit, 48 typically developing children and their parents returned to complete the extinction recall phase of the study. Of the original sample, 42 (87.5%) were Caucasian, 3 (6.3%) were African-American, 1 (2.1%) was Asian, and 2 (4.2%) were identified by their parent as "Other" ethnicities. There were 15 5-to-6 year olds (8 females; $M_{age}$

= 5.92; *SD* = .56), 15 7-to-8 year olds (9 females; $M_{age}$ = 8.03; *SD* = .64), and 18 9-to-10 year olds (10 females; $M_{age}$ = 10.07; *SD* = .51).

***Procedure***

Following the completion of the first laboratory visit, participants were invited to return to the laboratory to complete the extinction-recall phase. The time between the first and second laboratory visit was approximately three weeks (*M*= 19.77; *SD*= 8.20 days). Participants' subjective responses of their current levels of positive and negative affect to the CS+ and CS- were collected at set times throughout the second laboratory visit.

***The Extinction Recall Task***

Stimuli included the blue and yellow cartoon bells (i.e., CS+, CS-) used in the fear acquisition and extinction phases. Additionally, children were randomly presented with cartoon bell sequences of 11 different colored gradients ranging between blue and yellow (see Figure 2). Specifically, each stimuli corresponded to color gradients of 0% (CS-), 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100% (CS+). Each "morph" was randomly presented for 3000ms for a total of 8 trials with an inter-trial interval of 500ms. Following each stimulus presentation, participants were instructed to use a mouse to respond either "yes" or "no" to one of three questions: a) are you afraid of this bell? (i.e., threat appraisal); b) did the bell make a sound? (i.e., explicit memory); and c) is the bell yellow? (i.e., perceptual discrimination). Participants were instructed to answer each question based on their 'gut feeling' and to respond as quickly as possible. All responses and reaction times were recorded. The task was presented via E-Prime

computer software (PST Inc., Pittsburgh, PA). All stimuli were presented using a 17" IBM computer laptop with children seated 65 cm from the computer monitor during the task.

### *Subjective Ratings of Anxiety*

Participants completed subjective fear ratings and anxiety levels when viewing the CS+ and CS- using a ten-point Likert scale (1= none, 10= extreme). Similar to the first laboratory visit, this form was administered following the completion of the extinction recall phase and were comprised of questions such as "how scared are you of this blue bell?" and "how anxious were you when you saw the blue bell?"

### *Data Analysis*

Generalization effects for threat appraisal and explicit memory were analyzed using a 11 (morph type: 0% (CS-), 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100% (CS+) x 3 (age group: 5-6 year olds, 7-8 year olds, 9-10 year olds) repeated measures ANOVA. Subjective ratings and reaction times were analyzed using PASW (Version 20.0). Significant effects were detected at $\alpha = 0.05$ level. Post-hoc analyses were conducted using Bonferroni correction.

## Results

### *Fear Acquisition and Extinction across all Age Groups*

To test the validity of the Bell Conditioning task, we first analyzed children's contingency awareness during the 3 phases. A 3 (Phase: pre-acquisition, fear acquisition, extinction) x 2 (Stimulus type: CS+, CS-) analysis of variance across all age groups revealed a significant interaction, $F(2, 106) = 5.61$, $p = .00$ (see Figure 3). Follow-up *t*-tests showed greater

SCR (CS+>CS-) during fear acquisition ($t(53) = 2.20$, $p = .03$) compared to pre-acquisition ($t(53) = -.71$, $p > .05$) and extinction ($t(53) = .22$, $p > .05$). Similar analysis of variance for subjective fear ratings yielded a significant interaction showing robust fear acquisition ($F(1.69, 89.54) = 11.08$, $p = .00$; see Figure 4). Follow-up *t*-tests revealed no effects during pre-acquisition ($t(53) = -.83$, $p > .05$) compared to fear acquisition ($t(53) = 4.47$, $p = .00$) and extinction ($t(53) = 3.30$, $p = .00$).

To further explore contingency awareness, as measured by SCR, trials were divided into early and late blocks for each phase. A 6 (Phase: early/late pre-acquisition, early/late fear acquisition, early/late extinction) x 2 (Stimulus type: CS+, CS-) revealed a significant interaction effect, $F(5, 260) = 2.47$, $p = .03$. Specifically, the second half of SCR trials during the fear acquisition block were significantly larger ($t(53) = 3.92$, $p = .00$) than the remaining blocks with as evidenced by no CS differences.

Next, to examine SCR over time, analysis for 11 trial bins x 2 stimulus type showed a significant interaction effect, $F(6.88, 358.07) = 2.01$, $p = .03$. In particular, children across all age groups showed greater SCR during trials 5 and 6 ($t(53) = 2.03$, $p = .04$) as well as 7 and 8 ($t(53) = 4.20$, $p = .00$) during the fear acquisition phase. The above findings suggest the Bell Conditioning task was successful in eliciting conditioned fear responses, as evidenced by both SCR and subjective ratings of fear, across 5-to 10 year old children.

***Developmental Differences in Fear Acquisition and Extinction***

Next, we tested developmental differences in fear acquisition and extinction as a function of age. The repeated measures ANOVA with age group as the between-subjects factor and phase and stimulus type as the within-subjects factor revealed a significant main effect of phase ($F(2,$

4) = 3.11, $p$ = .04) for SCR. No other significant main or interaction effects ($F$(4, 102) = .45, $p$ > .05) for age-group differences in SCR levels were detected across the three phases (see Figure 5). Analyses of subjective fear ratings revealed no main or interaction effect ($F$ (4, 102) = .220, $p$ > .05).

In order to assess potential age differences in fear learning and extinction, each age group was examined separately for SCR and subjective ratings (3 Phase: pre-acquisition, fear acquisition, extinction) x 2 (Stimulus type: CS+, CS-). SCR in 5-to-6 year old children (n=19) showed a main effect of phase that was significant at trend level ($F$(2, 36) = 2.87, $p$ = .06) suggesting increased SCR during fear acquisition but no differences in contingency awareness of CS. Similarly, subjective ratings for this age group showed a significant main effect of phase, $F$ (2, 36) = 4.74, $p$ = .01. No significant interaction effects emerged, suggesting that 5-to-6 year children's SCR and subjective ratings of fear did not change as a result of the CS+ and CS- during fear acquisition (see Figure 6).

Seven-to-eight year old children (n = 17) revealed no significant main effects or interactions ($F$ (2, 32) = .41, $p$ > .05) in SCR across the three phases (see Figure 7). In contrast, subjective ratings showed a significant interaction of phase x stimulus type, $F$ (2, 32) = 3.43, $p$ = .04, with children reporting greater subjective fear during fear acquisition ($t$(16) = 2.35, $p$ = .03) compared to pre-acquisition ($t$(16) = -1.00, $p$ > .05) and extinction ($t$(16) = 1.69, $p$ > .05). Findings in this age range indicate that participants increased in their subjective fear ratings but SCR did not show expected conditioned fear responses to the CS across the three phases.

Lastly, examination of SCR in 9-to-10 year old children (n = 18) showed a significant phase x stimulus type interaction, $F$ (2, 34) = 5.82, $p$ = .00 (see Figure 8). Participants showed greater SCR during fear acquisition ($t$(17) = 3.70, $p$ = .00) versus pre-acquisition ($t$(17) -.06, $p$ >

.05) and extinction ($t(17) = .57$, $p > .05$). Early and late analyses of phases showed a significant interaction effect, $F$ (5, 85) = 2.71, $p = .02$, during the second half of trials in the fear acquisition phase ($t(17) = 4.42$, $p = .00$) while no other effects were significant. Additionally, individual SCR for each trial yielded a significant interaction effect of 11 trial bins x 2 stimulus type, $F$ (10, 170) = 1.86, $p = .05$. Findings showed increased SCR during trials 5 and 6 ($t(17) = 3.17$, $p = .00$) as well as 7 and 8 ($t(17) = 2.43$, $p = .02$) during the fear acquisition phase. Subjective ratings also revealed a significant interaction, $F$ (2, 34) = 7.80, $p = .00$, in which 9-to-10 year old children reported greater fear during fear acquisition ($t(17) = 3.54$, $p = .00$) and extinction ($t(17) = 2.20$, $p = .03$) compared to pre-acquisition ($t(17) = 1.00$, $p > .05$). No other significant main or interaction effects for SCR and subjective ratings were obtained.

***Extinction Recall Task: Subjective Threat Appraisal***

To examine participants' subjective threat appraisal, a significant main effect of morph type, $F(10) = 7.71$, $p = .00$, was found (see Figure 9). The main effect of morph type yielded both linear, $F(1, 45) = 8.07$, $p = .00$, and quadratic gradients, $F(1, 45) = 16.09$, $p = .00$. In addition, there was an interaction effect of morph type x age group that was significant at trend level ($F(20, 450) = 1.50$, $p = .07$). Reaction times to threat appraisal responses across morph types showed no significant main or interaction effects.

***Extinction Recall Task: Explicit Memory***

Responses to explicit memory instruction for morph type revealed a significant main effect, $F(10) = 51.22$, $p = .00$, with linear [$F(1, 45) = 63.488$, $p = .00$] and quadratic ($F(1, 45) = 107.44$, $p = .00$) components (see Figure 9). A significant morph type x age group interaction

effect for explicit memory, $F(10) = 51.22$, $p = .00$, was found consisting of a linear slope [$F(2, 45) = 3.78$, $p = .03$]. Follow-up *t*-tests revealed significantly greater explicit memory for 9-to-10 year old [$t(14.84) = -2.22$, $p = .04$] and 7-to-8 at a trend level [$t(17.57) = -1.83$, $p = .08$] compared to 5-to-6 year old children. No significant main or interaction effects were found in explicit memory reaction times as a function of morph types.

## Discussion

The overall goal of the current study was to examine whether typically developing 5-to-10 year old children show differences in fear conditioning, extinction learning, and fear generalization as a function of age. First, we sought to test the feasibility of using a novel fear conditioning paradigm (e.g., cartoon bell paired with the loud alarm sound) to support fear conditioning among 5-to-10 year old children. SCR and subjective rating data revealed that all children showed robust differential fear conditioning to the CS+ compared to the CS- as well as successful extinction learning. These results appear to be consistent with previous research showing evidence of successful acquisition and extinction of CR among typically developing children within this age range (Neuman, Waters, Westbury, & Henry, 2008; Gao et al., 2010; Pattwell et al., 2012). Importantly, findings from this study highlight the potential utility of using this novel fear conditioning paradigm in the study of fear learning processes and extend the existing literature of fear learning in typically developing children.

While the current study did not reveal developmental differences at the group level, differences across the three phases of the study emerged when each age group was examined separately. Specifically, 5-to-6 year old children failed to show SCR or behavioral evidence of discrimination fear learning suggesting increased arousal levels to both CSs during fear

conditioning compared to pre-conditioning and extinction. Seven-to-eight year old children did not show differential SCR to the CSs across conditions but reported increased subjective fear during fear conditioning to the CS+ versus the CS- in comparison to pre-conditioning and extinction phases. Physiological and subjective measures of fear in 9-to-10 year olds demonstrated discriminative fear conditioning (CS+ > CS-) during fear conditioning. Following extinction, SCR showed no differences in CR while subjective measures of fear remained high to both CSs. Consistent with prior findings (Glenn et al., 2012), 9-to-10 year old children showed a linear fear generalization pattern in threat appraisal suggesting a greater ability to discriminate between gradations of the CS+ and CS- in older children. Even though fear learning was evidenced across all participants, contingency awareness (CS-UCS) and generalization effects significantly increased with age. One interpretation of these findings is that more complex forms of fear learning emerge and continue to develop as children approach adulthood (Britton, Lissek, Grillon, Norcross, & Pine, 2011; Glenn et al., 2012; Prattwell et al., 2012). Findings from the youngest age group may reflect early cortical development in brain regions associated to learning processes in children. Studies in fear conditioning using animal models have shown that early learning experiences and age significantly influence fear conditioning (Sevelinges, Sullivan, Messaoudi, & Mouly, 2007). While mature rats learn to avoid odors previously paired with shock, young infant rats at postnatal day 10 displayed approach behaviors to threat conditioned odors which allow infant rats to orient toward their mother's odor and subsequently facilitating mother-infant attachment (Sullivan, Landers, Yeaman, & Wilson, 2000). Additionally, extinction learning in infant rats has been found to involve amygdala activation whereas older rats involve activation in both amygdala and ventromedial prefrontal cortex regions (Kim & Richardson,

2010). This plasticity in young rodents suggests a sensitive period in which fear learning is suppressed and amygdala activation is reduced (for review see Landers & Sullivan, 2012).

While the mechanisms underlying fear learning processes in children are still unclear, it is possible that developmental differences result from changes in cortical maturation as well as structural connectivity across childhood. For instance, in a recent study researchers found a positive shift in amygdala and medial prefrontal cortex (mPFC) functional connectivity in young participants suggesting greater bottom-up amygdala activity to the mPFC during childhood while the inverse relation was found in older participants signaling a transition to top-down activity among these regions (Gee et al., 2013a). More importantly, the developmental switch from positive to negative functional connectivity was related to less amygdala reactivity and a decline in developmentally normative anxiety. Thus, rather than a failure to display discrimination learning, findings from the current study may reflect a possible developmental pattern resulting from changes in amygdala-prefrontal connectivity in which younger children show a general increase in arousal levels to emotionally salient information, irrespective of its contingency to the UCS.

To explore the malleability of sensitive periods in fear learning, researchers have also examined amygdala activation in response to threats as a function of corticosterone levels (CORT) and stress (Moriceau & Sullivan, 2004). Findings showed that low levels of endogenous CORT in young rats at PND 8 resulted in the inability to form learning associations related to aversive olfactory stimuli whereas stress-induced CORT at PND 12 resulted in expected amygdala activation and subsequent fear learning behaviors to aversive odors. Similar findings were observed with previously institutionalized children who had experienced early environmental stress as evidenced by maternal deprivation (Gee et al., 2013b). Results revealed a

negative shift in amygdala and mPFC structural connectivity among PI children suggesting amygdala hyperactivity and neural patterns indicative of a mature connectivity found typically among older subjects. Similar to the rodent study (Moriceau & Sullivan, 2004), these findings suggest that high levels of stress can trigger early amygdala maturation and fear learning behaviors. Taken together, studies examining young rodents and humans reveal that fear learning circuits may be particularly plastic during early development given that hypo-reactivity in amygdala activation may result in reduced discrimination to threatening stimuli.

Given that fear learning deficits characterize anxiety disorders in adults and children, it is unclear whether at-risk samples may exhibit similar behavioral and psychophysiology of fear learning compared to anxious populations. Several studies have begun to examine individual differences in psychophysiological responses to safe and threat cues in at-risk pediatric samples. For instance, 7-to-12 year old children with a parental history of diagnosed anxiety disorders showed enhanced startle reactivity to threat cues compare to controls and children diagnosed with anxiety (Waters, Craske, Bergman, Naliboff, Negoro, & Ornitz, 2008). Alternatively, enhanced startle magnitudes to safe cues have been found in 7-year-old children (Barker, Reeb-Sutherland, & Fox, 2012) and adolescents (Reeb-Sutherland et al., 2009) characterized with temperamental behavioral inhibition, a fearful emotional and physiological reactivity to novelty empirically linked to the development of anxiety disorders (see Degnan & Fox, 2007; Pérez-Edgar & Fox, 2005). Specifically, these findings suggest potential disruptions in fear generalization to safety cues in at-risk samples and have important implications for identifying potential psychophysiological processes that characterize childhood anxiety disorders. One possible moderator to play a role in the link between at-risk samples and later anxiety is fear learning processes. Coupled with the above discussion, the current study highlights the

importance of age related changes in fear learning processes particularly for when perturbations may confer risk for later anxiety disorders. Thus, a promising avenue for future research in fear learning may be to examine at-risk children.

Findings from the present study should be interpreted in the context of some study limitations. The lack of support for conditioning differences at the group level could reflect sample size limitations within each age group. Additionally, participants were almost exclusively normatively developing Caucasian children, reducing generalizability of findings to more diverse or clinical populations. Second, data from this study relied on a cross-sectional design which limits our ability to make inferences about developmental trajectories of fear learning. Thus, future longitudinal studies in fear learning, extinction, and fear generalization will be necessary. Third, SCR was used to index fear conditioning and extinction in the study. This measure is a non-invasive method used to measure autonomic levels of arousal and has been shown to successfully capture CR in adults and children (Solimon et al., 2010; Waters et al., 2008). While this measure captures subtle changes in physiological arousal, it does not differentiate between positive and negative valence of emotional states. In contrast, fear potentiated startle measures increased startle reflexes during affective states of fear to an aversive stimulus, an index of individual differences in negative emotional reactivity, and has been postulated as a possible physiological biomarker for an anxiety endophenotype (Grillon et al., 1994; Morgan et al., 1995; Merikangas, Avenevoli, Dierker, Grillon, 1999; Barker et al., 2012). As physiological data was collected only during the first visit (i.e., fear conditioning, extinction) and not the second visit (i.e., fear generalization) of the study, future research would benefit from collecting continuous physiological measures across both visits. Thus, the addition of multiple psychophysiological

measures in fear learning studies may provide more meaningful information regarding autonomic measures of fear learning in developing populations.

This study was among the few to examine physiological and behavioral measures to capture fear learning, extinction, and fear generalization patterns in a sample of 5-6, 7-8, and 9-10 year old children. Additionally, the use of a novel fear conditioning paradigm proved to be feasible across a wide age range. The present work is important for laying the groundwork on empirical and theoretical questions about how children learn and adjust their behavioral responses to their environment and could greatly contribute to the understanding of the etiology of pediatric anxiety disorders as well as inform new approaches to early detection.

***Figure 1.*** Diagram of the experimental task during three phases: pre-acquisition, fear acquisition, and extinction.

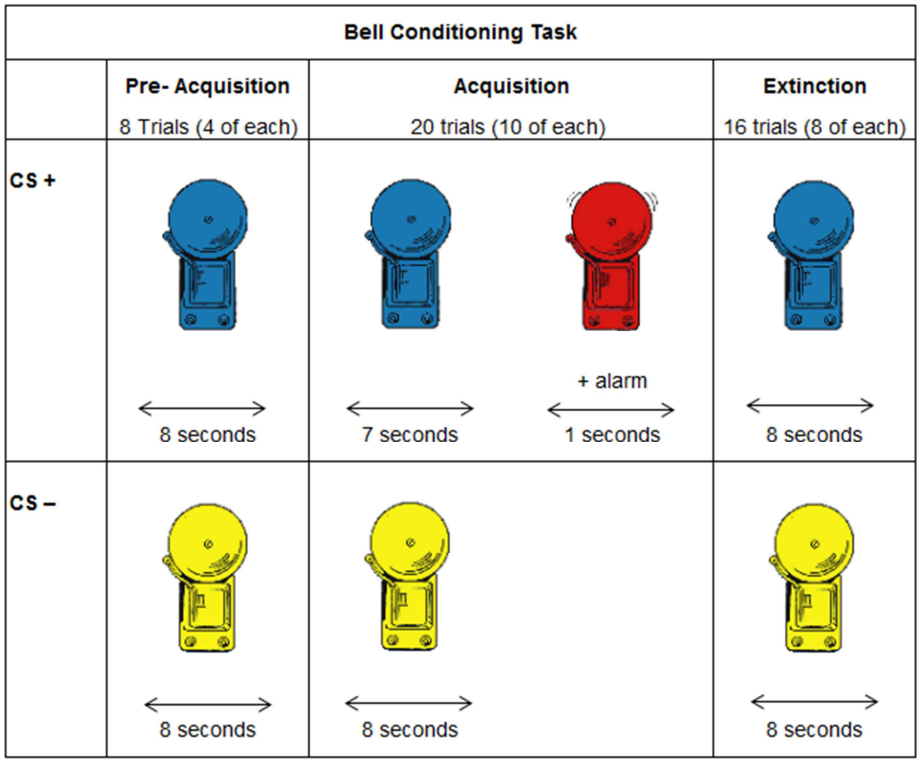
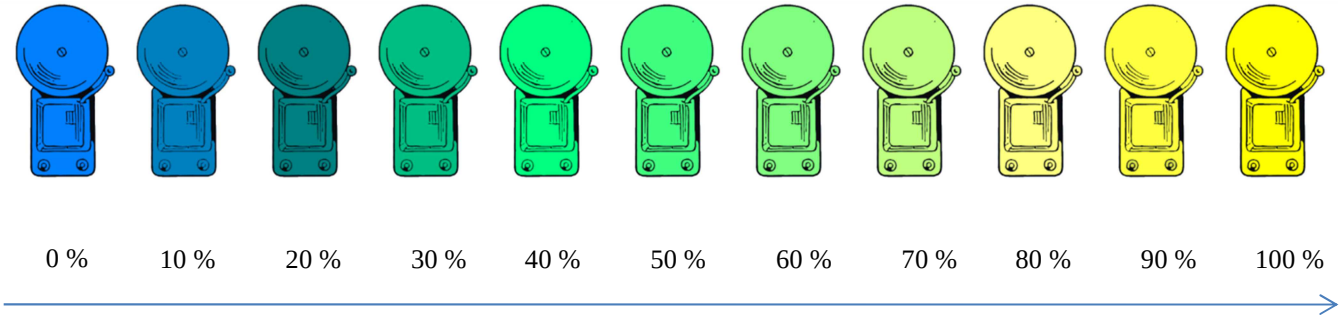


***Figure 2.*** Example of generalization stimuli during extinction recall task.



0 % 10 % 20 % 30 % 40 % 50 % 60 % 70 % 80 % 90 % 100 %

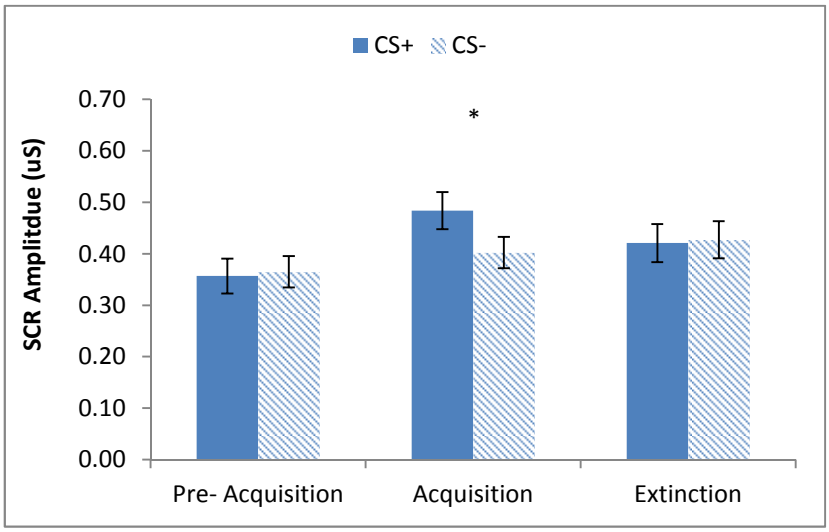
**CS-** **CS+**

Threat Appraisal: Are you afraid of this bell?

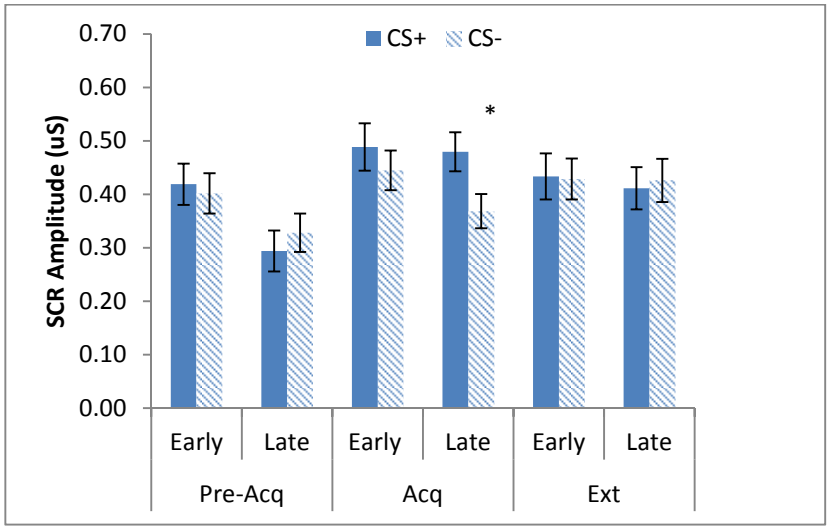
Explicit Memory: Did this bell make a sound?

***Figure 3.*** SCR measures of fear acquisition and extinction across age groups.

A.




B.



C.

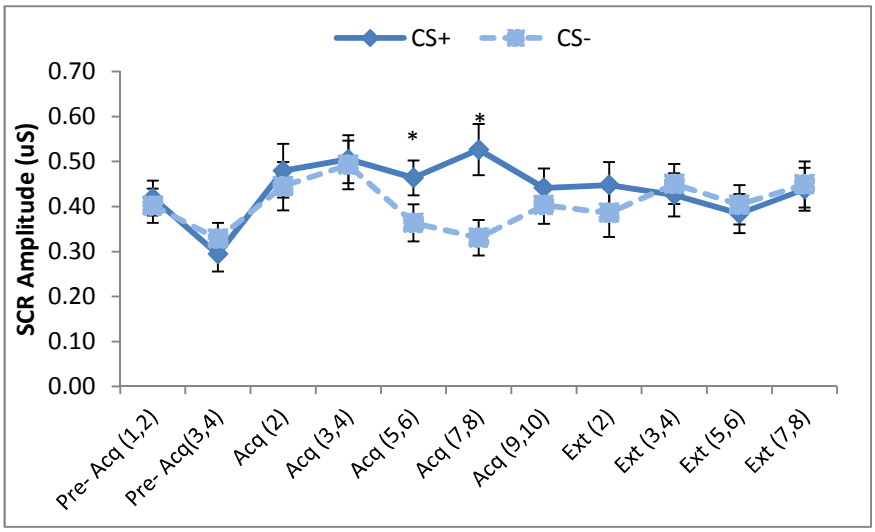

CS+
CS-
SCR Amplitude (uS)
0.70
0.60
0.50
0.40
0.30
0.20
0.10
0.00
*
*
Pre- Acq (1,2)
Pre- Acq(3,4)
Acq (2)
Acq (3,4)
Acq (5,6)
Acq (7,8)
Acq (9,10)
Ext (2)
Ext (3,4)
Ext (5,6)
Ext (7,8)

***Figure 4.*** Subjective ratings of fear acquisition and extinction across age groups.

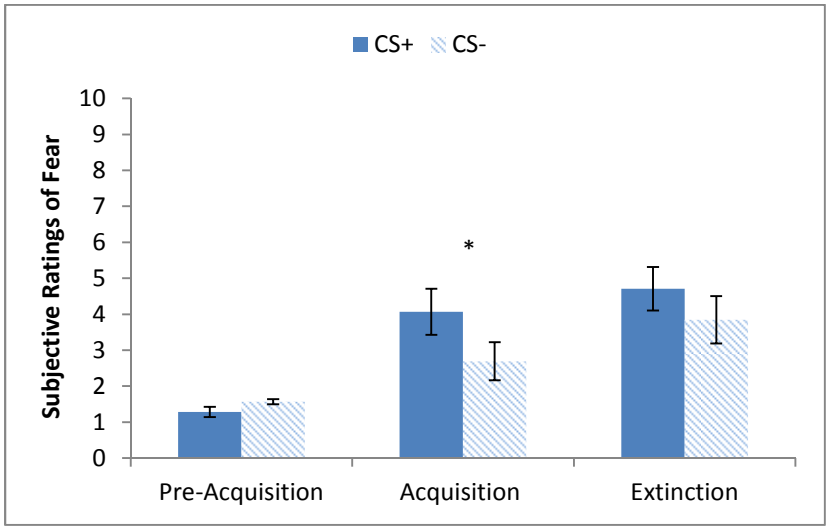
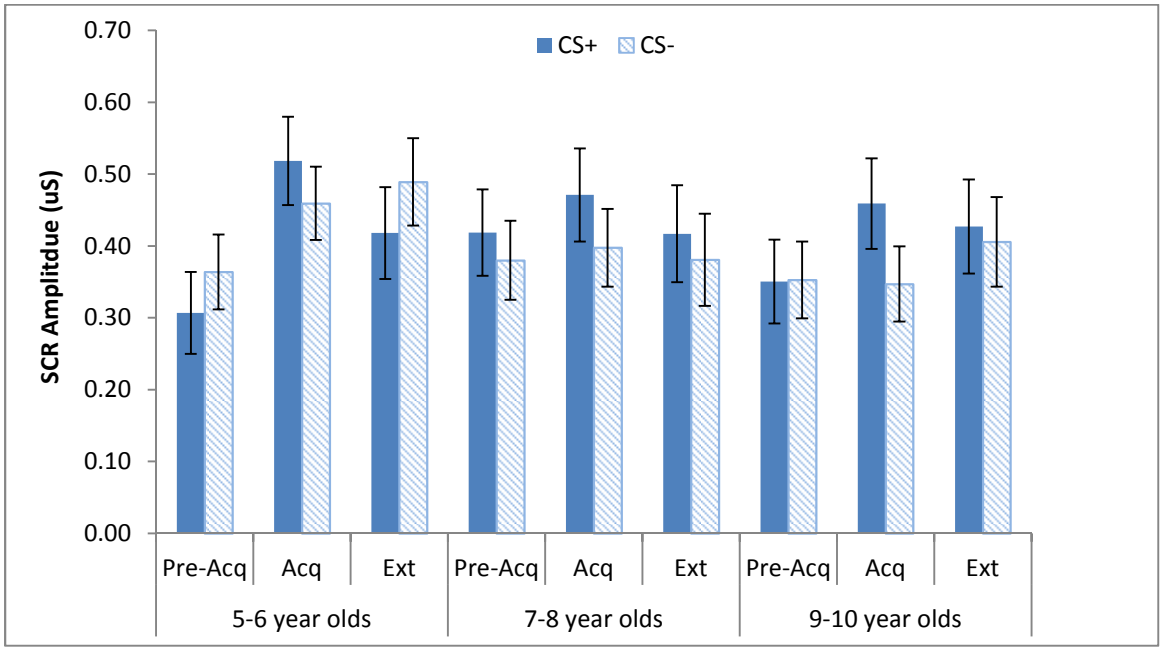


***Figure 5.*** SCR measures and subjective ratings of fear acquisition and extinction as a function of age.

A.




B.

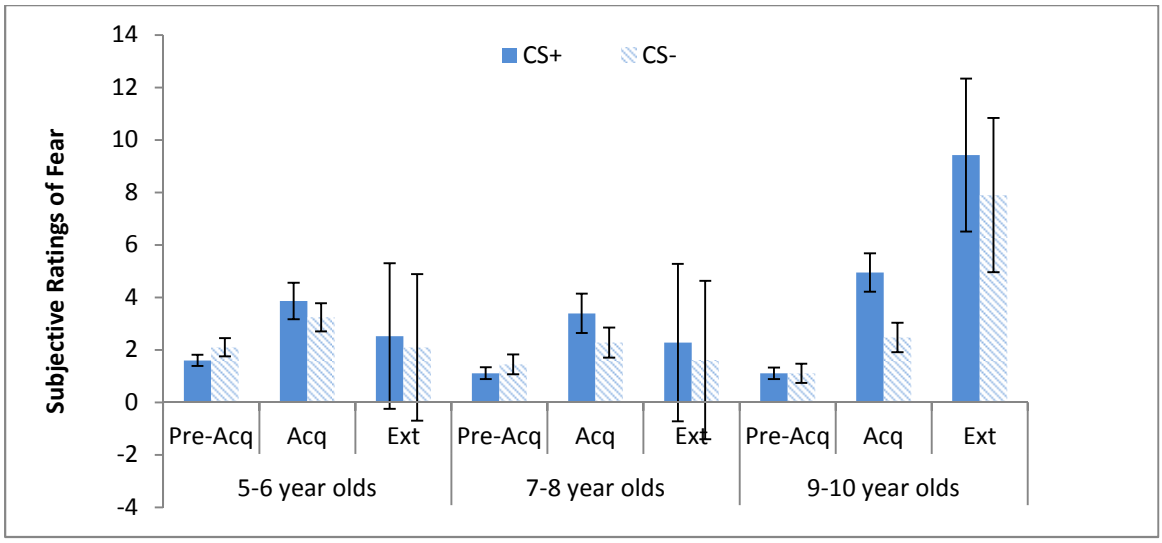
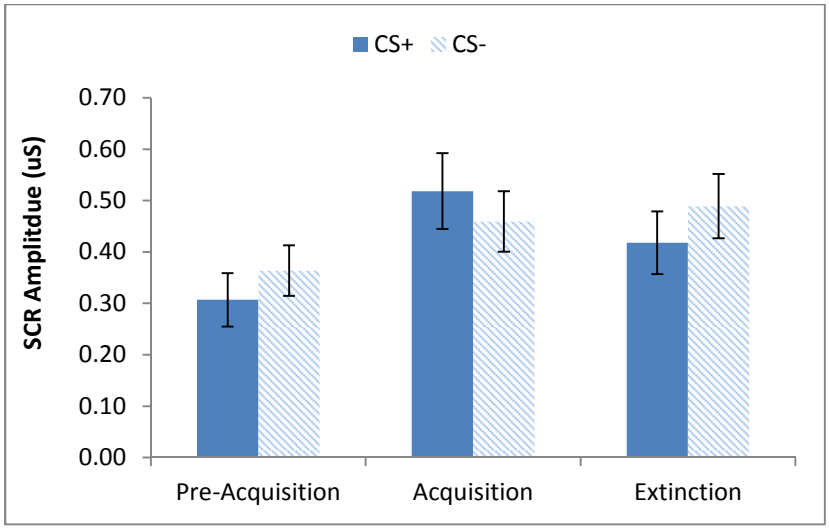


***Figure 6.*** SCR measures and subjective ratings of fear acquisition and extinction among 5-to-6 year old children.

A.




B.

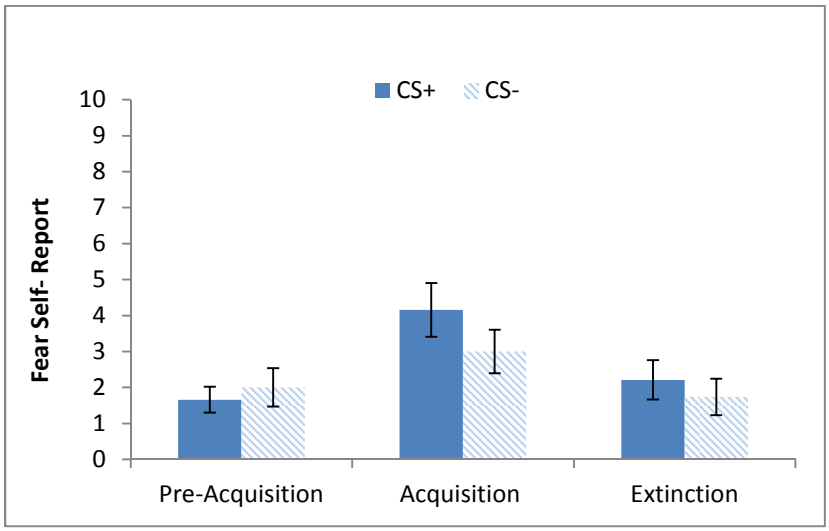
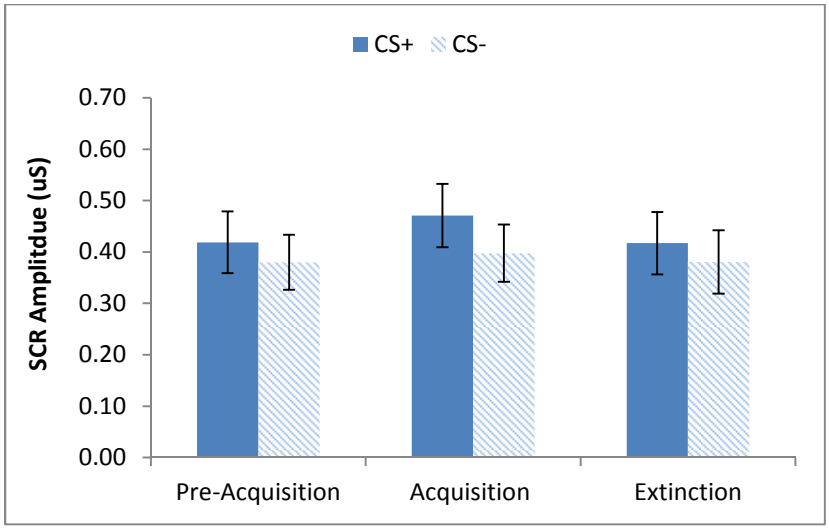


***Figure 7.*** SCR measures and subjective ratings of fear acquisition and extinction among 7-to-8 year old children.

A.




B.

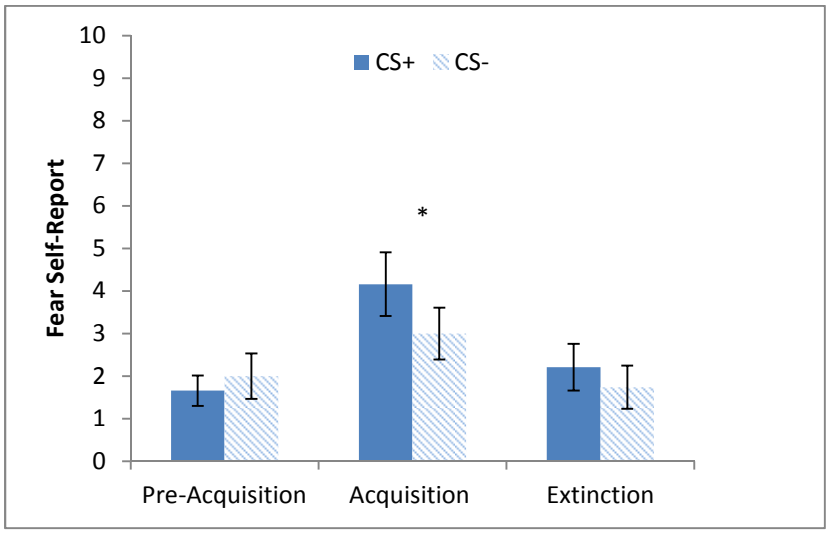
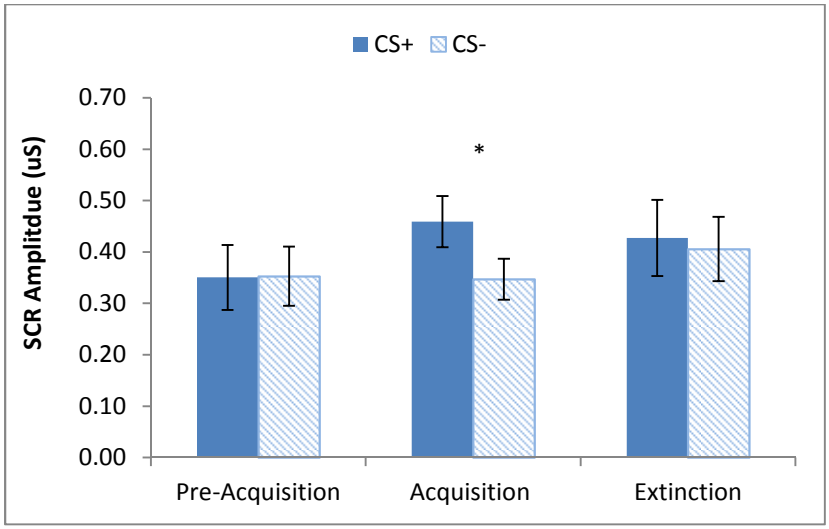
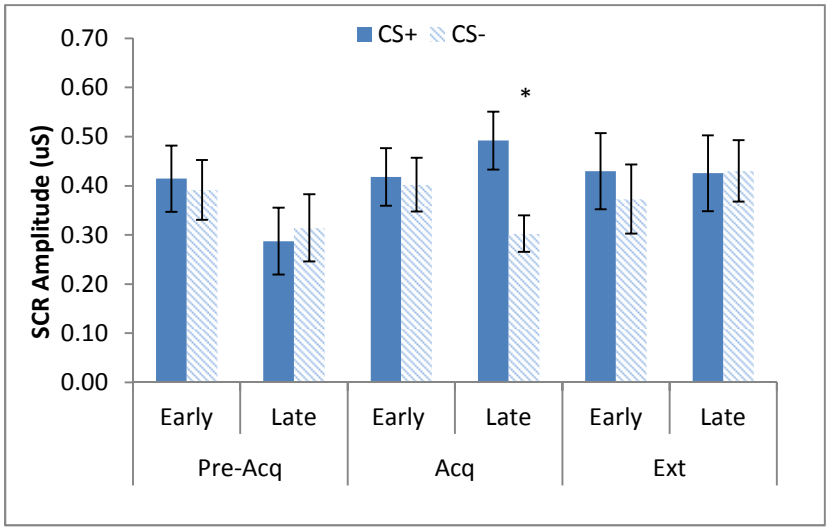


***Figure 8.*** SCR measures and subjective ratings of fear acquisition and extinction among 9-to-10 year old children.

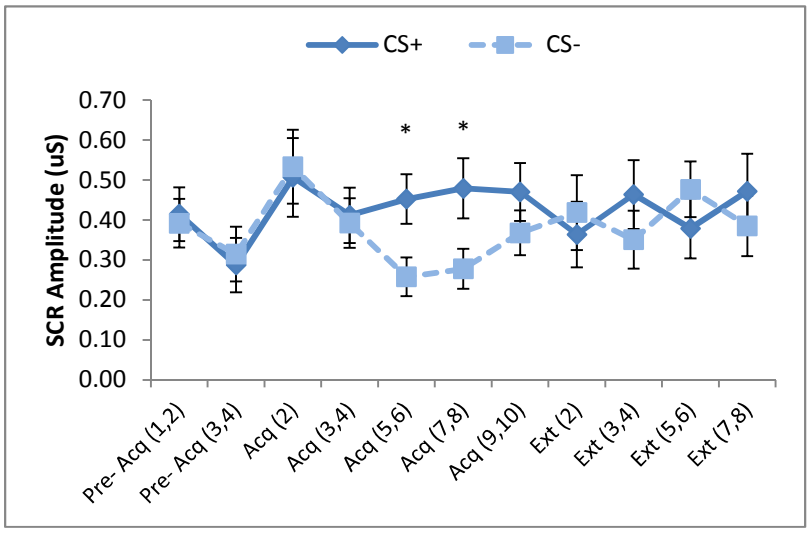
A.




B.



C.


CS+
CS-
SCR Amplitude (uS)
0.70
0.60
0.50
0.40
0.30
0.20
0.10
0.00
*
*
Pre- Acq (1,2)
Pre- Acq (3,4)
Acq (2)
Acq (3,4)
Acq (5,6)
Acq (7,8)
Acq (9,10)
Ext (2)
Ext (3,4)
Ext (5,6)
Ext (7,8)

D.

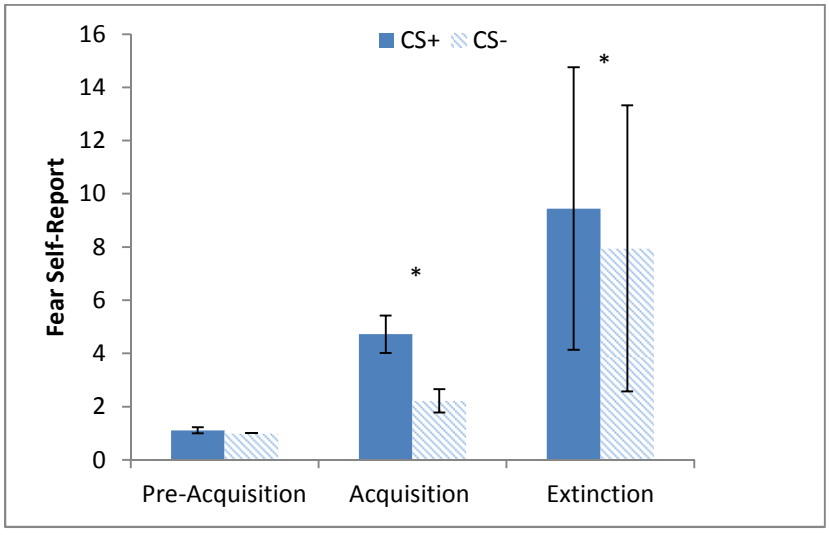
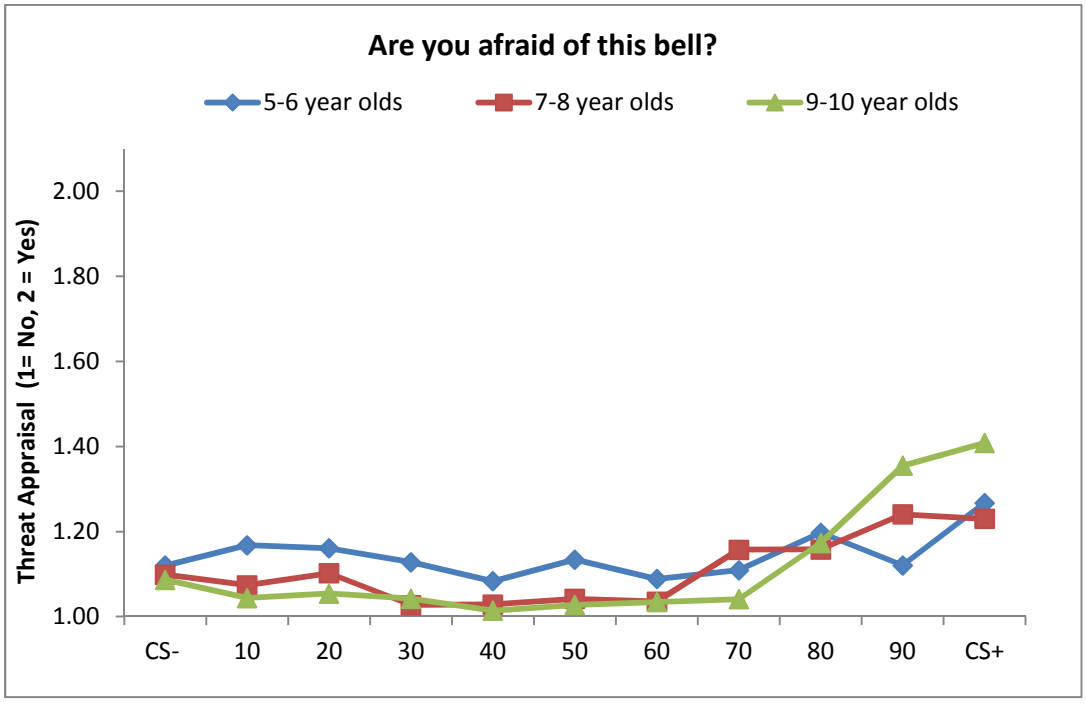
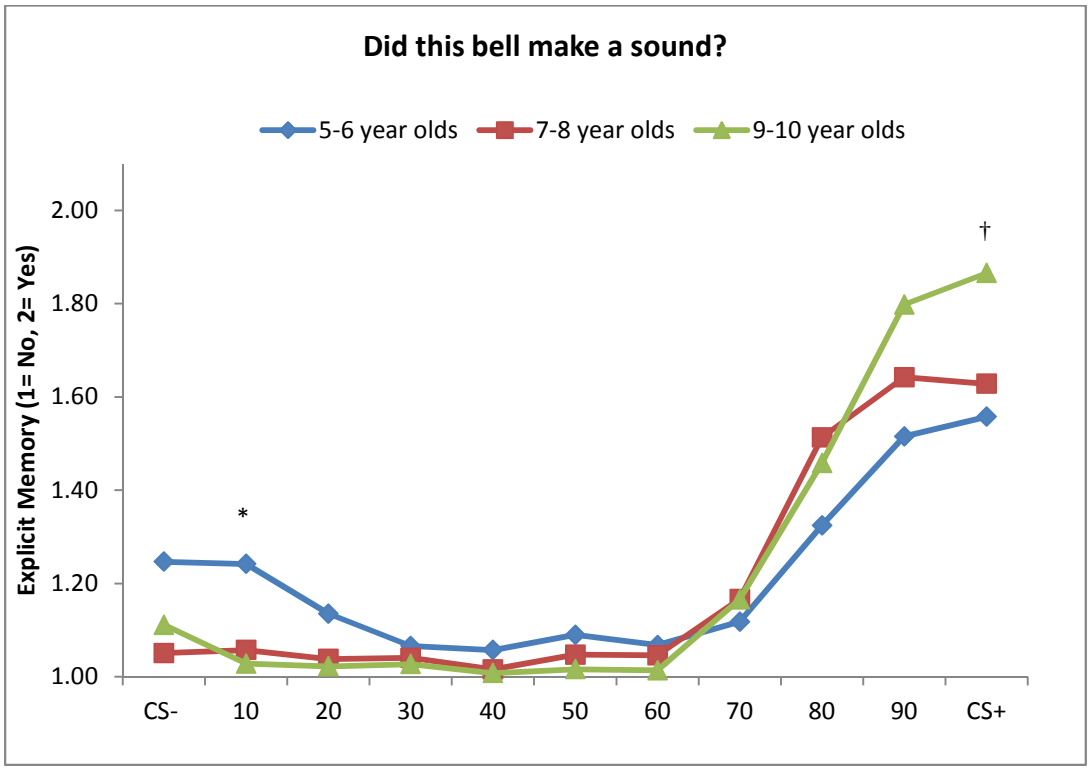

CS+
CS-
Fear Self-Report
16
14
12
10
8
6
4
2
0
*
*
Pre-Acquisition
Acquisition
Extinction

***Figure 9.*** Responses for threat appraisal and explicit memory responses across all age groups

A.




B.



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