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A developmental analysis of threat/safety learning and extinction recall during middle childhood

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ABSTRACT

The current study examined developmental changes in fear learning and generalization in 54 healthy 5–10-year old children using a novel fear conditioning paradigm. In this task, the conditioned stimuli (CS+/CS−) were two blue and yellow colored cartoon bells, and the unconditioned stimulus was an unpleasant loud alarm sound presented with a red cartoon bell. Physiological and subjective data were acquired. Three weeks after conditioning, 48 of these participants viewed the CS−, CS+, and morphed images resembling the CS+. Participants made threat–safety discriminations while appraising threat and remembering the CS+. Although no age-related differences in fear learning emerged, patterns of generalization were qualified by child age. Older children demonstrated better discrimination between the CS+ and CS morphs than younger age groups and also reported more fear to stimuli resembling the CS+ than younger children. Clinical implications and future directions are discussed.

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Introduction

Middle childhood is a crucial period for studying fear learning given age-related changes in memory abilities (Ofen, 2012; Riggins, 2014), brain structures that support fear learning and extinction (Gee et al., 2013; Ghetti & Bunge, 2012; Gogtay et al., 2004), and prevalence of fear-based internalizing disorders such as social anxiety disorder (Kessler, Berglund, Demler, Jin, & Walters, 2005). Although classic studies suggest that human infants manifest relatively mature capacities for fear learning by conditioning (Watson & Rayner, 1920), more recent work in humans (see Shechner, Hong, Britton, Pine, & Fox, 2014, for a review) and animals (e.g., Kim & Richardson, 2010; Rudy, 1993) finds that this capacity may change subtly throughout development. However, given methodological difficulties in studying fear learning among children, relatively little is known about how fear conditioning develops during middle childhood. Even less is known about fear extinction and recall of extinguished fears during this period. To address these difficulties, we developed a novel paradigm designed to assess the development of fear conditioning, extinction, and extinction recall in young children.

The literature on human fear learning corroborates findings in animals of the early emergence of the acquisition of fear (Pattwell et al., 2012), although relatively little is known about the development of this process, particularly in young children. The few available developmental studies of human fear conditioning indicate that children as young as 3 years show evidence of fear acquisition (Gao, Raine, Venables, Dawson, & Mednick, 2010), with discrimination between an aversively conditioned stimulus and a neutral stimulus (CS+ > CS−) gradually improving with age (Gao et al., 2010; Glenn et al., 2012). This increased discrimination ability with age continues into adulthood and is associated with distinct developmental patterns of neural activity during fear learning (Lau et al., 2011).

Although learned fear memories are persistent, their expression can be inhibited through new learning that a threatening stimulus in the past is now safe. Experimentally, this process of extinction learning is modeled by repeatedly presenting the CS+ without the aversive unconditioned stimulus (UCS) so that it no longer elicits a fear response. Developmental changes in fear extinction learning have also been observed across species. In particular, fear extinction in both humans and rodents is selectively attenuated during adolescence relative to children and adults (Pattwell et al., 2012). Based on these few studies, findings support that young children are 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 compared with adults. Taken together, age-related differences in fear and safety learning seem to emerge as more complex forms of learning continue to mature and interact with changes in neural circuitry.

The capacity for remembering and maintaining threat–safety discrimination also improves with age. Extinction recall, the retention of extinction over time, quantifies this more complex form of discrimination, although work in young children remains limited. Recent work suggests two possible means for detecting developmental change in fear learning during extinction recall. First, Lau and colleagues (2011) assessed participants' awareness of danger and found that adolescents showed reduced discrimination of threat and safety cues when rating fear during conditioning compared with adults. Second, extending this work, two studies combined a focus on awareness and on subtle discrimination by creating a generalization gradient using morphs that mixed features of the extinguished CS+ and CS− cues (i.e., generalization stimuli, GS) at extinction recall. These two studies demonstrated age differences in generalization (Britton et al., 2013; Glenn et al., 2012). However, across these three studies, the youngest participants were 8 years old. Prominent theories suggest that fear conditioning contributes to individual differences in anxiety because individuals differ in the extent to which they experience fear generalization (LeDoux, 1998; Lissek et al., 2014). Thus, there is a need for research that uses these methods in younger children. The aim of the current study was to test whether generalization gradients observed in older children differ in younger children.

Few studies have focused on fear generalization processes during middle childhood due to methodological limitations (i.e., UCS stimulus selection). Because it provides the strongest learning, most research in adults uses electric shock as a UCS, which is inappropriate for use in pediatric populations (Pine, Helfinstein, Bar-Haim, Nelson, & Fox, 2009). Some work with children has attempted to use milder aversive stimuli, such as a mildly aversive sound UCS (Gao et al., 2010; Neumann, Waters, & Westbury, 2008; Pattwell et al., 2012), which is more tolerable but not sufficiently intense to elicit individual differences in anxiety (Pattwell et al., 2012). To produce robust conditioning and study individual differences in fear conditioning across development, a more aversive yet ethically appropriate UCS is needed.

Attempts to find a middle ground have not been wholly successful. Investigators have used a fearful face coterminating with an aversive auditory scream as a UCS in youths (Britton et al., 2013; Glenn et al., 2012; Lau et al., 2008, 2011). Although potent at eliciting individual differences in fear, many youth abort the task because they find it too aversive (Britton et al., 2013). The large number of youth discontinuing the task due to the aversiveness of the UCS prompted the creation of a new task designed to elicit fear while remaining tolerable for sensitive populations, including young children (Shechner et al., 2015).

We examined fear conditioning and extinction recall in 5–10-year old children using colored cartoon bells as the CS and a loud alarm sound as the UCS. The goal was to examine developmental differences in fear conditioning, extinction learning, and response gradients at extinction recall among three age groups—5–6-year olds, 7–8-year olds, and 9–10-year olds—following previous work on the development of fear conditioning (Britton et al., 2013; Glenn et al., 2012; Lau et al., 2008, 2011; Pattwell et al., 2012). We collected skin conductance and self-report data to test three hypotheses based on prior studies of children in the literature (Britton et al., 2013; Glenn et al., 2012; Lau et al., 2008, 2011). First, we expected children across all age groups to show fear conditioning and extinction. Second, we expected that, with increasing age, children would show better discrimination between danger cues (CS+) and safety cues (CS−) and enhanced fear extinction. Third, we predicted enhanced conditioned fear generalization in younger children compared with older children.

Method

Participants

Families were recruited through mailings and advertisements from the Washington, D.C., metropolitan area in the eastern United States. Participants who verbally assented and whose primary caregivers gave written consent were enrolled in the study. Study procedures were approved by the University of Maryland institutional review board. The paradigm consisted of two visits. Visit 1 included the fear conditioning and extinction phases. Visit 2 included the extinction recall phase. For each visit, families were compensated for their time with toys valuing $20.

A total of 63 children between 5 and 10 years of age participated in the fear acquisition and extinction procedures. Four children discontinued participation when they became anxious (three 5–6-year olds and one 9–10-year old), leaving 59 children who contributed data. Children were divided into three age groups: 5–6 years (mean = 5.91 ± 0.57 years, range = 5.03–6.90), 7–8 years (mean = 8.04 ± 0.58 years, range = 7.07–8.96), and 9–10 years (mean = 10.07 ± 0.51 years, range = 9.18–10.95). From these 59 children, data from 5 children were excluded from skin conductance (SCR) analysis because of technical problems. Therefore, 54 children were included in the final SCR analysis for Visit 1. Of these participants, 48 children returned for Visit 2 (see Table 1 for demographics).

Table 1
Demographic characteristics of children in the analyses.

	n			Race/ethnicity				
Task and age group	Total	Female (%)	Age in years [*M* (*SD*)]	Hispanic (%)	Caucasian (%)	African American (%)	Asian (%)	Other (%)
Fear acquisition and extinction								
5–6 years	20	50.00	5.91 (0.57)	0	68.4	15.8	5.3	10.5
7–8 years	20	55.00	8.04 (0.58)	0	88.2	5.9	0	5.9
9–10 years	19	57.00	10.10 (0.51)	0	94.4	0	0	5.6
Extinction recall								
5–6 years	15	53.33	5.92 (0.56)	0	80.0	13.3	6.7	0
7–8 years	15	60.00	8.03 (0.64)	0	86.7	6.7	0	6.7
9–10 years	18	55.56	10.07 (0.51)	0	94.4	0	0	5.4

Materials

During the fear conditioning and extinction phases of the experiment (Visit 1), one cartoon blue colored bell and one cartoon yellow colored bell served as the CS. The UCS was an unpleasant loud alarm sound presented at 95 dB for 1 s concurrently with a red bell figure. The CS+ was followed by the UCS according to an 80% reinforcement schedule. During the extinction recall phase (Visit 2), stimuli included the blue and yellow cartoon bells (i.e., CS+ and CS−) used in the fear acquisition and extinction phases. In addition to these two stimuli, children also saw cartoon bell sequences of nine different bells with various colored gradients ranging between blue and yellow. Together, the stimuli formed a color continuum in 10% increments (see Fig. 1).

Procedure

Similar to previous studies, a differential fear conditioning and extinction paradigm was used (Britton et al., 2013; Lau et al., 2011). Fear conditioning consisted of a pre-acquisition phase and an

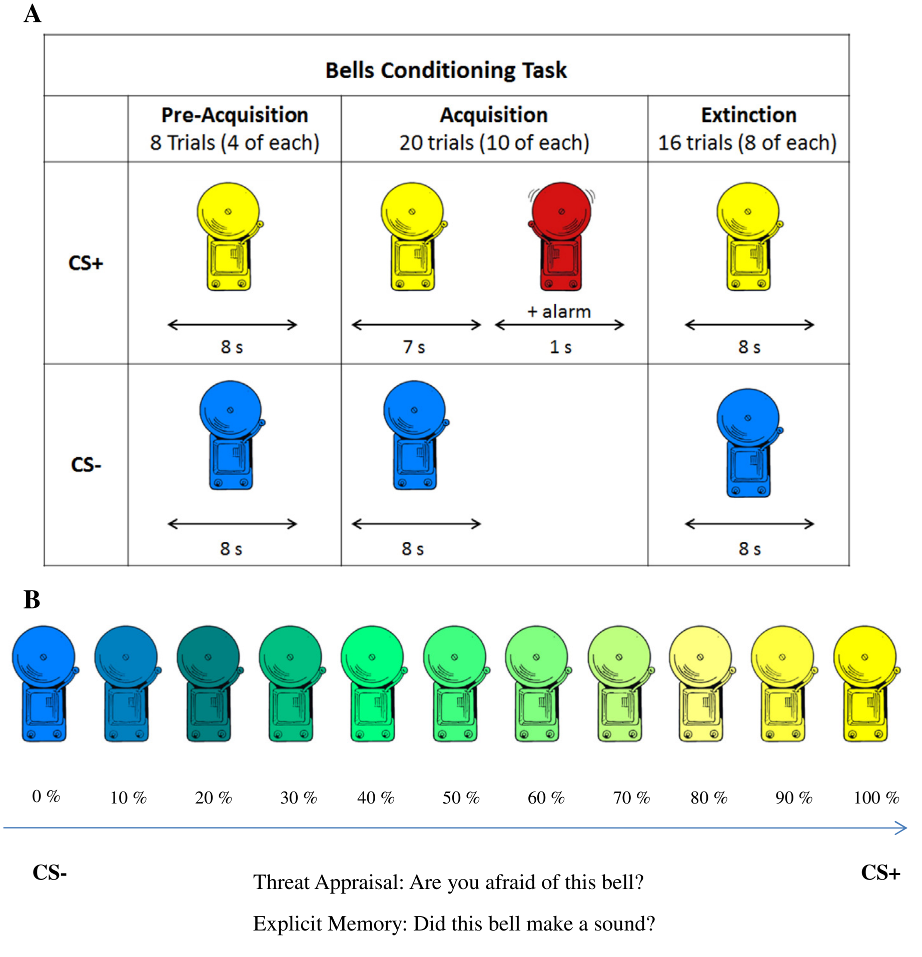

Fig. 1. Schematic depiction of the pre-acquisition, acquisition, and extinction phases of the conditioning paradigm (A) and the generalization stimuli during the extinction recall task (B). CS, conditioned stimulus.

acquisition phase (see Fig. 1 and online supplementary material for more details). SCR and self-reported anxiety were used to assess fear acquisition and extinction.

Three weeks after fear conditioning and extinction (mean = 19.77 ± 8.2 days), participants returned to the laboratory for the extinction recall procedure (Table 2). At this visit, participants viewed the CS+ and CS− and nine additional morphed images consisting of different blends of the CS− and CS+ (Fig. 1). All morphed images were presented in blocks, and the task instructions varied across blocks. The task was composed of (a) a threat appraisal component that assessed subjective fear levels and (b) a memory component that assessed explicit episodic memory of the extinguished fear. Within each block, children were instructed to endorse "yes" or "no" via a mouse click in response to one of two questions: (a) "Are you afraid of this bell?" (threat appraisal) and (b) "Did this bell make a sound?" (explicit memory). A third question asked children about the color of the bell but was not used in the current analysis and is not discussed further here. Children were instructed to answer each question based on their "gut feeling" and to respond as quickly as possible. Responses and reaction times were recorded. Each morphed stimulus was randomly presented for 3000 ms with an intertrial interval of 500 ms. A total of eight blocks of morphed images were administered for each question.

Children subsequently completed subjective fear ratings when viewing the CS+ and CS− using a 10-point Likert scale (1 = *none*, 10 = *extreme*).

Data analysis

Two dependent variables measured fear during the pre-conditioning, conditioning, and extinction phases: (a) average SCR level during each phase and (b) self-reported fear to each bell following each phase. SCR for each CS+ and CS− was determined by the difference between base and peak amplitudes within 1 to 7 s after the stimulus onset. SCR scores were normalized using a square root transformation and range corrected (SCR/SCR_{max}). SCR_{max} was determined as a participant's largest SCR to a CS during the entire conditioning session. SCR and self-report measures were each submitted to a separate repeated measures analysis of variance (ANOVA), with phase (preconditioning, conditioning, extinction) and stimuli (CS+, CS−) as within-subject factors and age group (5–6 years, 7–8 years, 9–10 years) as a between-subject factor. Significant results for ANOVAs were further examined for specific effects using post-hoc analysis. For all analyses, statistical significance was set to $\alpha = .05$.

Effects of threat appraisal and threat memory conditions during extinction recall were analyzed using a mixed model regression analysis (SPSS Version 20). Linear and quadratic trends of participant responses to the 11 morphed images—0% (CS−), 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, and 100% (CS+)—were examined for each task instruction as well as the interaction between linear and quadratic trends and age group (5–6 years, 7–8 years, 9–10 years). In addition, a repeated measures ANOVA with 11 response times (RTs) for each morph as a within-subject factor and age group as a between-subject factor was used to examine differences in RT across different morph levels for the two task conditions. Significant effects are reported at an $\alpha = .05$ threshold.

Results

Fear conditioning

SCR results

No significant three-way interaction emerged, $F(4,102) = 0.180$, $p = .94$, $\eta_p^2 = .007$. However, the phase by stimulus type interaction was significant in the entire sample, $F(2,102) = 3.31$, $p = .040$,

Table 2
Mean time between acquisition/extinction and extinction recall of children in the analyses.

Age group	Extinction recall total	Time between Visit 1 and Visit 2 [*M* (*SD*)]
5–6 years	15	18.86 (8.94)
7–8 years	15	23.00 (8.21)
9–10 years	18	17.83 (7.08)

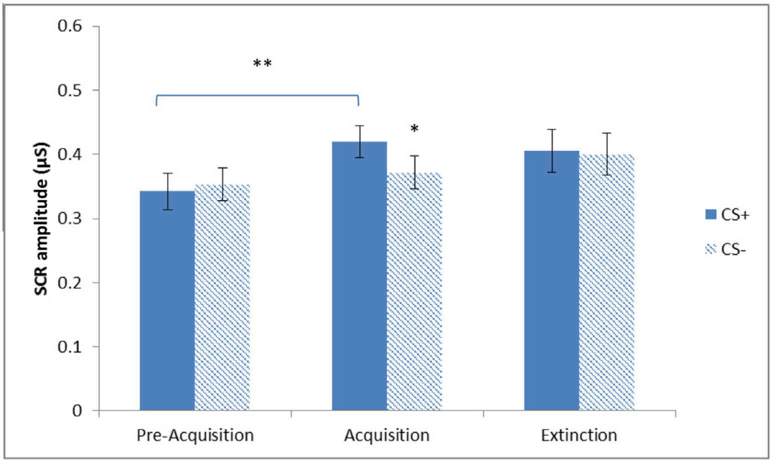

Fig. 2. SCR measures of fear acquisition and extinction across all age groups. *$p < .05$; **$p < .005$.

$\eta_p^2 = .06$ (see Fig. 2). Follow-up contrasts revealed a significant difference in SCR amplitude to the CS+ compared with the CS− after fear acquisition, $t(53) = 2.91$, $p = .005$, $d = 0.40$, but not after pre-acquisition, $t(53) = -0.41$, $p = .68$, $d = 0.06$, and extinction, $t(53) = 0.48$, $p = .63$, $d = 0.07$.

Self-report ratings

Children's fearfulness ratings of the bell stimuli are presented in Fig. 3. A repeated measures ANOVA yielded a significant main effect of phase, $F(2,112) = 22.72$, $p < .001$, $\eta_p^2 = .289$, a main effect of stimulus type, $F(1,56) = 13.27$, $p = .001$, $\eta_p^2 = .192$, and a phase by stimulus type interaction, $F(2,112) = 9.21$, $p < .001$, $\eta_p^2 = .141$. Follow-up contrasts revealed no difference between CS+ and CS− during pre-acquisition, $t(58) = -1.24$, $p = .221$, $d = 0.16$, whereas ratings were greater for the CS+ relative to the CS− during both fear acquisition, $t(58) = 3.55$, $p = .001$, $d = 0.46$, and extinction, $t(58) = 3.392$, $p = .001$, $d = 0.44$. No three-way interaction with age group was observed, $F(4,112) = 0.460$, $p = .734$, $\eta_p^2 = .016$, although a stimulus type by age group interaction emerged. $F(2,56) = 3.26$, $p = .046$, $\eta_p^2 = .104$, with 9–10-year olds reporting the highest levels of subjective fear for the CS+ across all phases (10.37 ± 5.98) compared with 5–6-year olds (8.18 ± 4.80) and 7–8-year olds (6.95 ± 4.84).

Extinction recall

Subjective threat appraisal

A linear and a quadratic pattern of self-reported rating were observed for threat appraisal (linear: $B = 0.068$, $SE = 0.013$, $t(422.14) = 5.38$, $p < .001$; quadratic: $B = -0.010$, $SE = 0.002$, $t(234.25) = -6.41$, $p < .001$). In addition, a significant interaction between age group and morph^2 emerged, with the younger age group ($B = 0.006$, $SE = 0.002$, $t(234.24) = 2.84$, $p = .005$) and middle age group ($B = 0.005$, $SE = 0.002$, $t(234.24) = 2.02$, $p = .044$) reporting less fear to stimuli resembling the CS+ than the oldest age group. RTs to threat appraisal responses across morph types showed no age group or age group by morphs interaction effects (all $ps > .454$) (see Fig. 4A).

Explicit memory

A linear and a quadratic pattern of self-reported rating were observed for threat memory (linear: $B = 0.128$, $SE = 0.016$, $t(411.65) = 7.85$, $p < .001$; quadratic: $B = -0.021$, $SE = 0.002$, $t(328.92) = -11.22$, $p < .001$). As is evident in Fig. 4, an age effect was apparent in regard to greater associations between the UCS and morphed images with higher CS+ features. Specifically, the older age group remembered the UCS–CS+ association better than the younger age group ($p = .028$), and a trend in the same direction emerged between the older age group and the middle age group ($p = .087$).

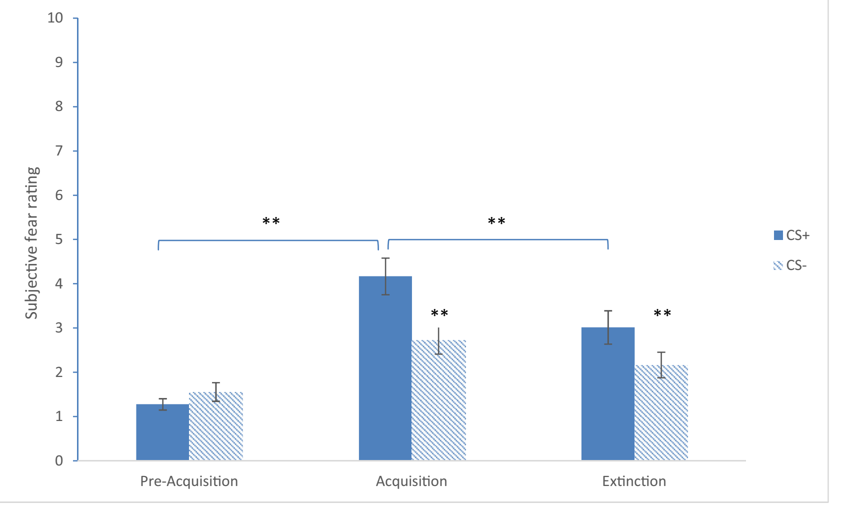

Fig. 3. Subjective ratings of fear acquisition and extinction across all age groups. $^{**}p < .005$.

In addition, a significant interaction between age group and linear morph emerged, with only the middle age group ($B = -0.057$, $SE = 0.024$, $t(411.65) = -2.35$, $p = .020$), but not the younger age group ($B = -0.007$, $SE = 0.024$, $t(411.65) = -0.29$, $p > .050$), being significantly different from the oldest age group. Finally, a significant interaction between age and morph^2 emerged, with the older group demonstrating better discrimination than the younger age group ($B = 0.005$, $SE = 0.003$, $t(328.92) = 1.97$, $p = .049$) and the middle age group ($B = 0.007$, $SE = 0.003$, $t(328.92) = 2.60$, $p = .010$). Furthermore, the similarities in linear and quadratic trends between the older and younger age group are likely related to the higher reported associations between CSs with lower CS+ features and the UCS, evident only in the younger age group. Specifically, the younger age group reported greater association between the CS 10% and the UCS compared with the middle age group ($p = .027$) and the older age group ($p = .008$). No significant age group or age group by morph interaction effects were found in explicit memory RTs (all $ps > .400$) (see Fig. 4B).

Discussion

This study examined developmental changes in patterns of fear learning with a novel task that examined fear conditioning, extinction, and extinction recall during middle childhood. Three main findings emerged. First, the task was tolerated by the majority of youth (i.e., only 6.3% discontinued vs. 49% anxious youths and 14% healthy youths in a previous study; Britton et al., 2013). Robust and comparable levels of fear conditioning and extinction were manifest at all ages (5–6 years, 7–8 years, and 9–10 years). Second, although all children showed quadratic fear generalization patterns during self-reported threat appraisal, older children reported more fear to stimuli resembling the CS+ than the two younger age groups. Third, during explicit recall, 9–10-year olds showed better discrimination and memory than younger children.

Based on physiological and self-report data, our results demonstrated that, overall, children could differentiate the CS+ from the CS− during fear acquisition. The magnitude of this effect was consistent with that in previous studies with healthy adults (Lau et al., 2011; Shechner et al., 2015) and typically developing children within this age range (Gao et al., 2010; Neumann et al., 2008; Pattwell et al., 2012), and we did not detect any age differences in fear acquisition or extinction learning. This result may reflect the fact that the oldest participants in our study were 10 years of age; previous age differences have mostly been observed in adolescents, who display attenuated fear extinction learning compared with children and adults (Pattwell et al., 2012).

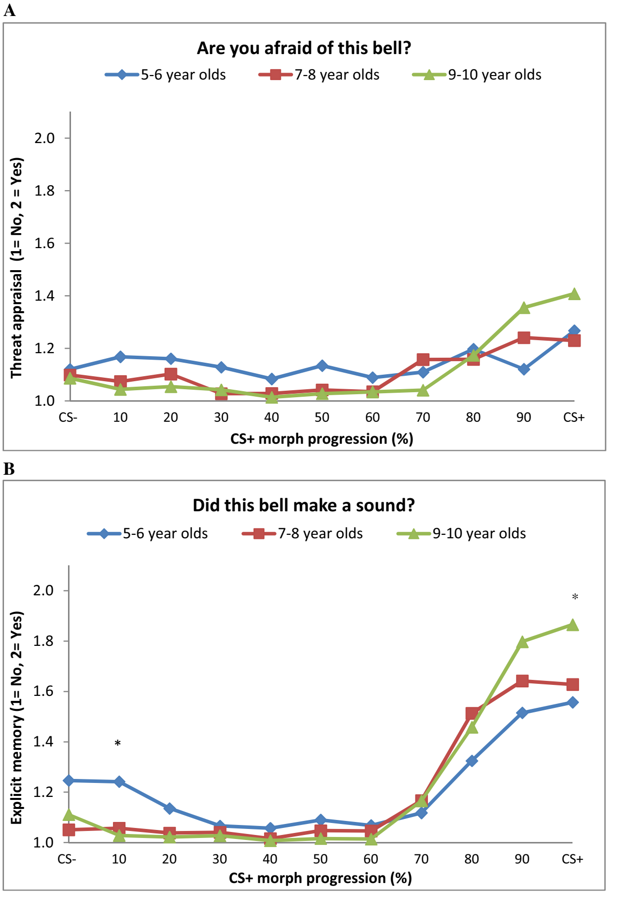

Fig. 4. Responses for threat appraisal (A) and explicit memory responses (B) across all age groups. $^{*}p < .05$.

Importantly, our findings highlight the potential utility of using this paradigm to extend the existing literature regarding fear learning in typically developing children. Such future research might probe the discrimination of generalization gradients at extinction recall in younger age groups. When appraising the threat of conditioned and morphed stimuli 3 weeks after fear conditioning, during extinction recall, children in the current study showed a quadratic response pattern (i.e., increasing from CS– to CS+), similar to previous work in adults (Britton et al., 2013; Hajcak et al., 2009; Lissek et al., 2010) and older youth (Britton et al., 2013; Glenn et al., 2012).

Of note, generalization patterns in threat appraisal among our oldest age group (9–10 years) differed from physiological responses in 8–10-year olds in Glenn and colleagues' (2012) study. In that study, based on the screaming lady task (Britton et al., 2013), the authors examined age differences in fear generalization among healthy 8–13-year olds by measuring responses to conditioned stimuli using one generalized stimulus, a 50% morphed blend of CS+ and CS faces. Older children (11–13 years) exhibited a linear fear generalization pattern, increasing from CS– to CS+. Unlike in our current study, however, 8–10-year olds in Glenn and colleagues' (2012) study displayed larger responses to the CS– compared with the GS. Several methodological differences between the two studies may explain the differing results. Our study (a) assessed threat appraisal during generalization with explicit verbal self-report, not fear potentiated startle; (b) used nonsocial cartoons as stimuli, not faces; and

(c) included a 3-week delay period between extinction and extinction recall, rather than no delay. Future research should attempt to understand the factors that might account for the distinct findings.

In the current study, developmental differences emerged in long-term retention of fear extinction at extinction recall. Specifically, explicit recall of the CS–UCS contingency (i.e., contingency awareness) and generalization effects improved significantly with age. The oldest age group in the current study was more likely to discriminate between gradations of the CS+ during the generalization phase (i.e., increasing from CS− to CS+) than both of the younger age groups. In contrast, the youngest children did not exhibit this pattern and instead displayed larger responses to the CS− compared with the GS. These patterns in older children, compared with younger children, may demonstrate a greater capacity to use their memory of the conditioning experience to recognize relatively subtle differences among fear-related gradations across CS types. Therefore, the current data suggest that fear recollection and generalization abilities, enabling children to distinguish similar-appearing stimuli, emerge and continue to develop as children approach adulthood (Britton et al., 2013; Glenn et al., 2012; Pattwell et al., 2012). Such findings in children resemble some findings in rodents, where data increasingly show that subtle forms of fear recollection continue to develop in a graded fashion from weaning age into adulthood (Kim & Richardson, 2010; Rudy, 1993). However, other findings, based largely on psychophysiological measures, suggest discontinuities in development. Such findings suggest that fear extinction and related processes develop in a nonlinear progression, such that adolescents show diminished abilities relative to preadolescents and adults (Pattwell et al., 2012). Therefore, it will be informative to extend the current work with this paradigm to include adolescent participants.

The observed age-related improvement in explicit recall supports the notion that memories are more fragile and vulnerable to disruption in younger children than in older children (Peterson, Warren, & Short, 2011). Both episodic memory (Riggins, 2014) and declarative memory (Drummey & Newcombe, 2002) have been shown to increase during middle childhood, with particularly rapid gains in episodic recall between 5 and 7 years (Riggins, 2014). The development of explicit memory formation during middle childhood is associated with age-related growth of activations in specific dorsolateral prefrontal cortex regions (Ofen et al., 2007). A promising future avenue for research will be to directly compare the developmental trajectories of "affect-related" and "neutral" memories, for instance, by comparing children's performance on an extinction recall task with performance on another item-based episodic task.

Limitations

Although the current study contributes to the growing number of fear learning studies by comparing young children on a fear conditioning and extinction recall task, several limitations should be noted. First, the sample size was relatively small, which may have limited our ability to observe age-related group differences in fear conditioning and extinction. Small sample size also prohibited our ability to test for potential relations between conditioning and recall, which may be relevant given associations observed in previous work (Glenn et al., 2012). However, despite the modest sample size the study acquired a considerable amount of data from more than 50 conditioning and extinction recall sessions in young children. Second, data from this study are cross-sectional, thereby limiting our ability to make inferences about developmental trajectories of fear learning. Future longitudinal studies in fear learning will be necessary. A third limitation relevant to sample selection is that all participants who completed the fear conditioning and extinction phases successfully were included in the analysis irrespective of whether they learned the CS–UCS contingency or showed differential conditioning as indexed by SCR. Although this approach allows findings to better represent the overall population, it could reduce our ability to detect some between-group developmental differences. Lastly, other limitations relate to methodological constraints of data acquisition. Physiological data were collected only during the first visit (i.e., fear conditioning and extinction) and not the second visit (i.e., extinction recall) of the study; future research would benefit from collecting continuous physiological measures across both visits. We also did not obtain trial-by-trial measurement of self-reported fear. Unlike SCR, self-report was collected only after participants completed the entire conditioning and extinction phases. A trial-by-trial measure can differ from a pre to post phase measure (Lipp, Oughton, & LeLievre, 2003) in that it is less reliant on memory and may be a relevant distinction for subjective self-report.

Conclusions

The current work is important for laying the groundwork for understanding the intersection between development and anxiety. Results from the current study indicate that the bell conditioning task is both potent in eliciting fear responses and tolerable for young children. Specifically, these findings suggest developmental differences during extinction recall and have important implications for identifying potential psychophysiological processes that characterize childhood anxiety disorders.

Acknowledgment

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jecp.2016.01.008.

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