Published in final edited form as:

Depress Anxiety. 2015 April ; 32(4): 277–288. doi:10.1002/da.22318.

FEAR CONDITIONING AND EXTINCTION IN ANXIOUS AND NONANXIOUS YOUTH AND ADULTS: EXAMINING A NOVEL DEVELOPMENTALLY APPROPRIATE FEAR-CONDITIONING TASK

Tomer Shechner, Ph.D.[1,*], **Jennifer C. Britton, Ph.D.**[2], **Emily G. Ronkin, B.A.**[3], **Johanna M. Jarcho, Ph.D.**[3], **Jamie A. Mash, B.A.**[3], **Kalina J. Michalska, Ph.D.**[3], **Ellen Leibenluft, M.D.**[4], and **Daniel S. Pine, M.D.**[3]

[1]Department of Psychology, University of Haifa, Israel [2]Department of Psychology, University of Miami, Florida [3]Section on Development and Affective Neuroscience, NIMH, Bethesda, Maryland [4]Section on Bipolar Disorder, NIMH, Bethesda, Maryland

Abstract

Background: Fear conditioning and extinction have been implicated in the pathogenesis of anxiety disorders. However, due to ethical and methodological limitations, few studies have examined these learning processes across development, particularly among anxious individuals. The present study examined differences in fear conditioning and extinction in anxious and nonanxious youth and adults using a novel task designed to be more tolerable for children than existing paradigms.

Methods: Twenty-two anxious adults, 15 anxious youth, 30 healthy adults, and 17 healthy youth completed two discriminative fear- conditioning tasks. A well-validated task paired a woman's fearful face with a scream as the unconditioned stimulus. The novel task paired a bell with an aversive alarm as the unconditioned stimulus. Self-reported fear, skin conductance response, and fear-potentiated startle eye blink were measured.

Results: Both tasks were well tolerated and elicited fear responses with moderate stability. Anxious youth and adults reported overall greater fear than healthy participants during the tasks, although no group differences occurred in discriminative fear conditioning or extinction, as assessed by self-report or physiology.

Conclusion: The novel bell-conditioning task is potent in eliciting fear responses but tolerable for pediatric and anxious populations. Our findings are consistent with prior studies that have shown comparable fear learning processes in anxious and nonanxious youth, but dissimilar from studies exhibiting between-group differences in extinction. Given the limited research on fear conditioning in youth, methodological issues and suggestions for future work are discussed. Depression and Anxiety 32:277-288, 2015.

[*]Correspondence to: Tomer Shechner, Psychology Department, University of Haifa, Mt Carmel, 3498838, Haifa, Israel., tshechner@psy.haifa.ac.il.

All authors report no conflict of interests.

Keywords

fear conditioning; extinction; pediatric anxiety; psychophysiology; SCR; fear-potentiated startle

INTRODUCTION

Considerable evidence suggests fear conditioning and extinction are fundamental to the expression of anxiety disorders. Studying fear conditioning and extinction across development has great relevance for identifying factors that moderate risk for, and resilience against, pediatric anxiety. The most common method for eliciting conditioned fear includes use of physically aversive unconditioned stimuli, such as electrical shock. Because ethical constraints intrinsic to studying youth preclude the use of physically aversive stimuli, there are relatively few studies of fear conditioning in children. One recent study used a visual/audio pairing of a woman screaming as an unconditioned stimulus to compare conditioned fear response in anxious and nonanxious youth and adults.[1] Although the results from this study suggest the presence of important developmental differences in fear conditioning, a large number of anxious patients and youth aborted the task prior to completion because they found the stimuli too aversive. This raises concerns about the tolerability of the scream-based fear-conditioning task for anxious patients and younger age groups. The current study examines differences in fear conditioning and extinction among anxious youth and adults using the scream-based task and a novel paradigm developed to elicit fear while remaining tolerable for sensitive populations.

Modest evidence suggests that anxious adults are more responsive to fear conditioning and extinction than healthy adults. However, these differences are more pronounced for single cue conditioning paradigms, where one conditioned stimulus (CS) is paired with an unconditional stimulus (UCS), than for discrimination conditioning paradigms, where one CS (CS+) but not another CS (CS−) is paired with the UCS.[2] Discrimination conditioning paradigms are imperative in the study of fear conditioning as they allow the examination of excitatory processes to danger cues (CS+), inhibitory processes to safe cues (CS−), and the ability to differentiate between the two stimuli. Findings from the few discrimination-based conditioning studies that compare fear response in anxious and nonanxious youth are somewhat inconsistent (for a review see[3]). Some report no group differences in fear conditioning,[1,4-6] whereas others report differences in extinction.[4,6,7]. The most consistent findings are that, regardless of age, anxious, relative to healthy individuals, exhibit elevated fear to both danger (CS+) and safe cues (CS-), but similar levels of differential fear learning.[1,4,5]

Conditioning paradigms in animals and adults typically employ an electric shock as the UCS, which is generally considered to be inappropriate for use in pediatric populations.[8,9] Thus, most studies in children have used mildly aversive sounds as a UCS,[8,10-12] which are more tolerable, but elicit lower levels of conditioned fear. To study fear conditioning across development, a more aversive UCS is needed to produce robust conditioning in both youth and adults. The “screaming lady task” has shown promise in this respect.[1,5,13,14] In this task, participants passively view neutral faces of two women, which serve as the conditioned

stimuli (CS). One neutral face, the CS+, but not the other neutral face, the CS-, is paired with a fearful expression and aversive auditory scream. Although potent in eliciting fear, the screaming lady task has two major limitations. First, similar to other studies in pediatric samples,[8,13] a relatively high proportion of youth discontinued. Although no adults discontinued fear acquisition, 49% of anxious youth and 14% of healthy youth discontinued due to high levels of fear.[1] Second, the use of social stimuli as a CS could instantiate fear in subjects prone to social anxiety, even in the absence of the UCS.

The current study of anxious and healthy adults and children utilizes a novel fear-conditioning paradigm, the "bell task," designed to address these limitations. In this task, two different color bells serve as CSs, and a red bell accompanied by an aversive alarm serves as the UCS. Stronger conditioning and extinction occur on tasks that use stronger, more potent, and biologically relevant stimuli.[3] One of the conditioning paradigms in the current study pairs a red bell with an aversive alarm; the other pairs a fearful face with an aversive scream. Thus, both pair sets of pictorial and auditory stimuli that also co-occur in genuinely dangerous situations in real life. As such, these two paradigms may be more aversive than paradigms using only an auditory UCS, possibly because the paradigms evoke reactions that occur to aversive events in children's daily lives.

Because the bell and screaming lady tasks share features, it is important to compare response to the two paradigms. Based on previous work, we expect anxious, relative to nonanxious participants, to show higher levels of fear to both CSs, but no differences in differential learning during fear conditioning or extinction. In order to validate the new task and examine within-subject consistency, each participant completed bell- and scream- based fear-conditioning and extinction paradigms. Results from the two tasks are compared across anxiety and age groups.

METHOD

PARTICIPANTS

Anxious youths and adults and their healthy counterparts participated in this study as paid volunteers. Written informed consent from adult participants and parents and written assent from youth participants were obtained. The National Institute of Mental Health Institutional Review Board approved all procedures.

Individuals were included in the study if they were medication free, physically healthy, and had an IQ > 70. All participants received a comprehensive psychiatric assessment. The Structured Clinical Interview for the DSM-IV-TR Axis I Disorders (SCID) was used for adults and the Kiddie Schedule for Affective Disorders and Schizophrenia (KSADS) was used for children. Parents and their children were interviewed separately. In line with previous clinical trials of anxiety disorders, child-parent discrepancies were resolved by the clinician as part of the diagnostic process used in previous research-based assessment enrolling children in treatment research.[15,16] Healthy youth and adults were included if they were free of any current Axis I disorders. Anxious youths and adults met DSM-IV-TR criteria for a current anxiety disorder. Other comorbid anxiety disorders or major depressive

disorder were permitted, however current or past diagnoses of PTSD or obsessive-compulsive disorder were excluded.

Using these procedures, 95 participants started the bell task, but three children aborted during conditioning, whereas 89 started the screaming lady task, but three children aborted during conditioning, and data for two additional adult participants were lost due to technical malfunction. Thus, 84 individuals completed both tasks. No prior data have been reported from either task in these participants, which included 15 anxiousyouths, 22 anxious adults, 17 healthyyouths, and 30 healthy adults. Clinical and demographic data are presented in Table 1. As expected, self-report and parent-report SCARED (Self-Report for Childhood Anxiety Related Emotional Disorder) scores were higher for anxious compared to nonanxious youth, $t(30) = 3.84$, $P < .001$, $t(29) = 6.50$, $P < .001$ (respectively). Similarly, anxious adults reported higher anxiety (indexed by Liebowitz Social Anxiety Scale scores) than their nonanxious counterparts, $t(53) = 5.67$, $P < .001$.

PSYCHOPHYSIOLOGICAL DATA ACQUISITION

Psychophysiological measures were used as one index of fear conditioning and extinction. Psychophysiological measures were collected continuously using PsyLab psychophysiological recording system (PsyLab SAM System Contact Precision Instruments, London, www.psylab.com) during the procedure.

Skin conductance response (SCR) was measured with two Ag/AgCl electrodes filled with nonsaline gel attached to the medial phalanx of the middle and ring fingers of the left hand. [17] SCR data were recorded using a sampling rate of 1,000 Hz.

Fear-potentiated startle (FPS)was measured with the eye blink star-tle reflex with two 6 mm tin-cup electromyography (EMG)electrodes filled with standard electrolyte solution placed under the subject's left eye along the cheekbone. A ground electrode was placed on the subject's left foream EMG data were obtained at a sampling rate of 1,000 Hz and filtered using an amplifier bandwidth of 30-500Hz.

PROCEDURES

Two fear-conditioning tasks were completed: the screaming lady task and the novel bell task. Details on the screaming lady task were described previously (e.g., [1]). The bell task follows the same conditioning procedures as the screaming lady task, but uses distinct audio and visual stimuli. Specifically, the CS+ and CS- are pictures of blue and yellow bells, instead of pictures of two women's faces, and the UCS is an aversive bell sound instead of a scream. The UCS consisted of a 1-s image of a red bell co-occurring with an aversive 95 dB alarm (see Fig. 1). These are corollaries of the fear expression and scream used as UCS in the screaming lady task. Participants completed each task on a separate visit, with at least 3 days between the two visits ($M_{\text{days between tasks}} = 25.64$, $SD = 7.60$). The order of the two procedures (screaming lady or bell task) was randomly determined.

In both tasks, the stimuli used for the CS+ and CS- were counterbalanced across participants. The CS+ and the CS- were presented for 7-8 s followed by a gray screen (intertrial interval; ITI) presented for 8-21 s (averaging 15 s). The CS+ was followed by the

UCS according to an 80% reinforcement schedule. Air puffs (4-10 psi of compressed air for 40 ms) were delivered to the forehead during the CS+, CS-, and ITI to measure FPS. Startle probes were delivered 5-6 s after CS onset to allow for measurement of SCR during the first 5 s of the trial.

The fear-conditioning tasks consisted of four different phases— habituation, preconditioning, fear conditioning, and extinction. In each phase, the CS+, CS-, and ITI were presented in different blocked counterbalanced order. Before the first phase, participants were told that they might see unpleasant pictures, hear unpleasant sounds, and feel an air puff on their forehead. They were also told that if they paid attention they may learn to predict when the sound will occur, but they were not informed of the UCS/CS +coupling.

During habituation, six startle probes without any visual stimuli were delivered. In the preconditioning phase, images of the CS+ and the CS- were presented four times each. During the fear-conditioning phase, images of the CS+ and CS- were presented 10 times each. The UCS was delivered eight times while paired with the CS+. Finally, in the extinction phase, the CS+ and the CS-were each presented eight times in the absence of the UCS.

Participants reported their levels of fear while viewing the CS+ and CS- using a 10-point Likert scale (1 = non, 10 = extreme) during preconditioning, and immediately following fear conditioning and extinction.

DATA ANALYSIS

Three dependent variables measured differences in fear for preconditioning, conditioning, and extinction phases across the two tasks: (1) self-reported fear to each bell/face following each phase; (2) average SCR level during each phase; (3) average EMG during each phase. SCR for each CS+ and CS- was determined by the difference between base-to-peak amplitude within 5 s after the stimulus onset. SCR scores were normalized using a square-root transformation. EMG data were rectified and smoothed using moving average with 20 ms windows. Startle response data for each participant was standardized using a T-score transformation.

Interclass correlation coefficients were used to test for temporal stability of the three dependent measures across the two tasks.

Self-report and SCR measures were each submitted to a separate repeated measures ANOVA, with 2 Tasks (bells, screaming lady) **x** 3 Phases (preconditioning, conditioning, extinction) ×2 Stimuli (CS+, CS-) as within-subject factors × 2 Age groups (youth, adults) × 2 Anxiety groups (anxious, nonanxious) as between-subject factors. A third ANOVA was conducted for EMG for which the stimuli factor included three levels (CS+, CS-, ITI). Task order (first, second) was added as another factor to each of these ANOVAs to examine for possible effects of task order. Significant results for ANOVAs were further examined for specific effects using post hoc analysis. For all analyses, statistical significance was set to $\alpha = .05$.

RESULTS

Four sets of results are presented. First, we report the number of participants who found the tasks too aversive to continue. Next, we present the interclass correlation analysis of the two tasks, which revealed correlations across tasks. Given these moderate to strong correlations, in a third analysis, fear responding was averaged across the tasks to increase power in this analysis. Therefore, the third analysis compares anxious and nonanxious youth and adults in fear conditioning and extinction, averaged across the two tasks (Phase × Stimuli × Anxiety group × Age group). Finally, we present the complete results from the six-way omnibus ANOVAs in the fourth analysis.

DISCONTINUATION OF TASKS

For the bell-conditioning task, two (10.5%) anxious children (one male and one female) and one (5.6%) nonanxious child (female) aborted during fear conditioning. For the screaming lady task, one (6%) anxious child (male) and two (10.5%) nonanxious children (1 male and 1 female) aborted the task during fear conditioning. Thus, discontinuation rates did not differ across the two tasks in either the anxious group, $\chi^2_{(1)} = .203$, $P = .653$, or the nonanxious group, $\chi^2_{(1)} = .307$, $P = .580$. Participants who terminated one task were excluded from the analysis.

Attrition rates among anxious and nonanxious youth also were compared to previous work using the screaming lady task in pediatric samples (Britton, 2013 #958). Thus, rates of discontinuation in individuals who completed the screaming lady task first in the current study were compared to anxious and nonanxious youth in the previous study.[1] Due to the small sample sizes, the Fisher exact test was used. Compared to seven nonanx- ious of 49 who aborted the task in the previous study,[1] the current study found 1 of 10 nonanxious subject aborted the scream task when it was delivered as the first- conditioning task. The Fisher exact test statistics was not significant, $P > .05$. This means that attrition rates for nonanxious samples were not different across the two studies for the scream among nonanxious youth. However, compared to 22 of 45 anxious youth that aborted the task in the previous study,[1] the current study found all eight anxious children successfully completed it. The Fisher exact test statistics indicated a significant difference between the studies, $P < .05$. Attrition rates were higher in the previous study among anxious children.

ASSOCIATION BETWEEN TWO TASKS

Interclass correlation analysis was conducted for each dependent measure, separately for CS + and CS-, across the two tasks. As evident in Table 2, across all groups, moderate to strong interclass correlation coefficients were found during conditioning and extinction for self-reported fear and SCR (ranging from .42 to .65), and modest correlation for EMG only during conditioning (.34-36) but not extinction.

AVERAGE RESPONDING IN ANXIOUS AND NONANXIOUS YOUTH AND ADULTS COLLAPSED ACROSS TASKS

ANOVA results for Phase × Stimuli × Anxiety group × Age group according to the three dependent variables (self-reported fear, SCR, EMG) collapsed across tasks are presented in Table 3.

SELF-REPORTED FEAR

As shown in Table 3, several findings across tasks emerged for self-reported fear. First, a main effect of anxiety emerged, $F(1, 76) = 10.11$, $P = .002$, partial $\eta^2 = .12$. Anxious participants reported higher levels of overall fear compared to their nonanxious counterparts. Second, the main effect of phase was significant, $F(2,152) = 71.32$, $P < .001$, partial $\eta^2 = .48$. Participants reported higher levels of fear during conditioning than preconditioning ($P < .001$) and extinction ($P < .001$). Finally, the main effect of stimuli was significant, $F(1, 76) = 49.45$, $P < .001$, partial $\eta^2 = .39$. Participants reported higher levels of fear to the CS+ than the CS-.

Both groups exhibited fear conditioning but not extinction based on self-reported fear. The Phase × Stimuli interaction was significant in the entire sample, $F(2, 152) = 21.38$, $P < .001$, partial $\eta^2 = .22$ (Table 3), as well as among anxious, $F(2, 72) = 11.36$, $P < .001$, partial $\eta^2 = .24$ and nonanxious participants, $F(2, 92) = 13.88$, $P < .001$, partial $\eta^2 = .23$ (Fig. 2A) considered individually. Follow-up contrasts revealed no differences between stimuli after preconditioning and significant differences in self-reported fear to the CS+ compared to the CS- after fear conditioning and extinction in both the anxious and nonanxious groups (all Ps $< .001$).

The ANOVA on self-reported fear with Phase-by-Stimuli-by-Anxiety group-by-Age group was not significant (Table 3).

SCR

Likewise, several findings emerged when examining SCR collapsed across tasks. The main effect of age was significant, $F(1, 73) = 24.28$, $P < .001$, partial $\eta^2 = .25$, indicating overall higher levels of SCR in youth than in adults. The main effect of stimuli was also significant, $F(1, 73) = 8.17$, $P = .006$, partial $\eta^2 = .10$. SCR levels were higher to the CS+ than to the CS-. Results also indicate that anxious participants did not differ from nonanxious participants in fear conditioning and extinction.

The Phase-by-Stimuli interaction was significant in the entire sample, $F(2, 146) = 8.48$, $P < .001$, partial $\eta^2 = .10$ (Table 3), as well as in the anxious, $F(2, 64) = 5.44$, $P = .007$, partial $\eta^2 = .15$, and nonanxious participants, $F(2, 82) = 3.36$, $P = .040$, partial $\eta^2 = .08$, considered individually (Fig. 2B). Follow-up contrasts revealed a significant difference in SCR to the CS+ compared to the CS- after fear conditioning (all Ps $< .008$), but not after preconditioning or extinction (all Ps $> .268$). Yet differential learning was observed only in youth but not in adults. A significant three-way interaction emerged for Phase-by-Stimuli-by-Age group, $F(2, 146) = 3.84$, $P = .024$, partial $\eta^2 = .05$ (Table 3). Examining each age group separately yielded a significant Phase-by-Stimuli interaction only in youth, $F(2, 50) =$

5.59, $P = .006$, partial $\eta^2 = .18$, but not in adults, $F(2, 96) = 1.92$, $P = .153$, partial $\eta^2 = .04$. Follow-up contrasts revealed a significant difference between the CS+ and the CS- in youth ($P = .002$) but not adults ($P = .109$).

The ANOVA on SCR with Phase-by-Stimuli- by-Anxiety group-by-Age group was not significant (Table 3).

FPS-EMG

As with SCR, the main effect of age was significant, $F(1, 74) = 4.89$, $P = .030$, partial $\eta^2 = .18$, with youth exhibiting higher overall levels of EMG compared to adults. In addition, a main effect of phase emerged, $F(2, 148) = 105.33$, $P < .001$, partial $\eta^2 = .59$, indicating a consistent habituation from preconditioning to conditioning ($P < .001$) and from conditioning to extinction ($P < .001$). Finally, the main effect of stimuli was significant, $F(2, 148) = 66.87$, $P < .001$, partial $\eta^2 = .48$. EMG levels were higher to the CS+ than to the CS-.

As with the self-report and SCR data, the Phase-by- Stimuli interaction was significant in the entire sample, $F(4, 296) = 4.36$, $P = .002$, partial $\eta^2 = .06$ (Table 3), as well as in the anxious, $F(4, 144) = 2.39$, $P = .05$, $n^2 = .06$, and the nonanxious participants, $F(4, 176) = 2.46$, $P = .047$, $\eta^2 = .05$ (Fig. 2c). Follow-up contrasts revealed a significant difference in EMG to the CS+ compared to the CS- after fear conditioning (all Ps $< .003$), but not after preconditioning (all Ps $> .418$) or extinction (all Ps $> .077$).

The ANOVA on EMG with Phase × Stimuli × Anxiety group × Age group was not significant (Table 3).

In sum, collapsed across the two tasks, both groups exhibited fear conditioning and extinction as indexed by SCR and EMG, and fear conditioning but not extinction based on self-reported fear; however, further analyses were conducted to understand differences between tasks.

TASK-SPECIFIC RESPONDING IN ANXIOUS AND NONANXIOUS YOUTH AND ADULTS

To understand task differences, we conducted six-way omnibus ANOVAS with 2 Tasks (bells, screaming lady) ×3 Phases (preconditioning, conditioning, extinction) × 2 Stimuli (CS+, CS-) × 2 Age groups (youth, adults) × 2 Anxiety groups (anxious, nonanxious) × 2 Task Order (bell task first, screaming lady task first) for each of the dependent variables (self-reported fear, SCR, EMG). In the next section, we present the additional effects of Task and Order and their interactions with the four other factors reported in the previous section (i.e., Phase, Stimuli, Age group, and Anxiety group).

SELF-REPORTED FEAR

A main effect of Task emerged, $F(1, 76) = 9.34$, $P = .003$, partial $\eta^2 = .11$, with participants reporting higher levels of fear from the screaming lady ($M = 3.00$, $SD = 0.17$) than the bell-conditioning task ($M = 2.51$, $SD = 0.13$). Thus, overall, reported levels of fear in the current study were higher in the screaming lady task than in the bell-conditioning task.

The three-way interaction for Task-by-Phase-by- Stimuli was significant, $F(2, 152) = 3.60$, $P = .030$, partial $\eta^2 = .045$, with the bell task eliciting greater differential learning (CS+ > CS-) during conditioning compared with the screaming lady task. This difference derives from higher level of reported fear to the CS- in the screaming lady task ($M = 3.30$, $SD = 2.40$) compared to the CS- in the bell task ($M = 2.54$, $SD = 2.00$), $t(83) = 2.68$, $P = .009$ in both children and adults.

Further, the three-way interaction for Task-by- Phase-by-Anxiety group also was significant, $F(2, 152) = 3.49$, $P = .033$, partial $\eta^2 = .044$ (Fig. 3). This interaction was decomposed according to anxiety group, yielding a significant Task-by-Phase interaction only among anxious subjects, $F(2, 72) = 5.21$, $P = .008$, partial $\eta^2 = .127$. This interaction derives from higher levels of self-reported fear of anxious participants to the social stimuli (CS) used in the screaming lady compared to bells even prior to conditioning, $t(36) = 3.31$, $P = .002$.

Finally, the five-way interaction for Task-by-Phase- by-Stimuli-by-Age group-by-Order was significant, $F(2, 152) = 5.82$, $P = .004$, partial $\eta^2 = .07$. Decomposing this interaction by isolating phase, age group, and order in successive steps revealed that the bell task elicited stronger differential learning effects (CS+ > CS-) in youth when the screaming lady task was delivered first, whereas in adults this differential effect was stronger when the bell task was administered first.

SCR

The ANOVA on SCR yielded a significant four-way interaction with Task-by-Phase-by-Stimuli-by-Age, $F(2, 146) = 3.87$, $P = .024$, partial $\eta^2 = .10$. This interaction was decomposed to a three-way Task-by- Phase-by-Stimuli interaction according to age group, resulting in nonsignificant results in both age groups. However, the interaction between Phase × Stimuli was significant in youth, $F(2, 50) = 5.59$, $P = .006$, partial $\eta^2 = .18$, but not in adults, $F(2, 96) = 1.92$, $P = .153$, partial $\eta^2 = 04$. Further, in youth, SCR was higher for CS+ compared to CS- during conditioning in both tasks (all $Ps < 0.029$), but there were no differences in SCR during extinction (all Ps > .297). Finally, a conditioning effect was obtained in adults only in the bell task $t(51) = 1.85$, $P = .046$, but not in the screaming lady task, $t(51) = .76$, $P = .450$ (Fig. 4). Results indicate that, among adult participants, the screaming lady did not elicit differential learning between the CS+ and the CS- as indicated by levels of SCR.

FPS-EMG

The ANOVA on EMG yielded a significant six-way interaction with Task-by-Phase-by-Stimuli-by-Age group-by-Anxiety group-by-Order, $F(4, 296) = 2.75$, $P = .029$, partial $\eta^2 = .036$. This interaction was decomposed by isolating it according to its factors. This process revealed the interaction to arise from one unusually strong finding in a particular group. Namely, the bell task elicited stronger differential learning effects (CS+ > CS−) in anxious youth, relative to other groups, when it was delivered after the screaming lady task.

DISCUSSION

This study is the first to examine différences between anxious and nonanxious youth and adults using a novel bell-based fear-conditioning task. The study also compared findings on this novel task to findings on a well- validated scream task. Both tasks were equally well tolerated in pediatric and clinical populations, and both tasks elicited fear responses with moderate stability, as indexed by correlations between the two tasks.

We first discuss the results for anxious and nonanxious youth and adults collapsed across tasks. We then discuss the specific findings that emerged for the two type of tasks used in the present study. Three main findings emerged from this set of results. First, anxious participants reported greater fear to both CSs than nonanxious participants. Second, comparable fear-conditioning and extinction effects were obtained in all groups (healthy and anxious, adults and children) on self-reported fear and FPS (EMG), but only in youth on SCR. Third, some differences across the two tasks were observed as a function of task delivery order.

One of the goals of this study was to validate a novel conditioning task using a bell as a UCS by comparing findings on this task with a previously used scream task. Specifically, compared to Britton et al.,[1] the attrition rates in the current study for both the screaming lady and the bell tasks were relatively low with few participants aborting either fear-conditioning task. This lower rate could be attributed to the relatively small sample size in both studies as well as some level of habituation to repeated administration of the tasks in the current study. Completion rates also were compared among youth who underwent the scream task first in the current study, relative to rates from the previous study.[1] In these contrasts, no differences emerged for the nonanxious group, but a significant difference did emerge for the anxious group. Attrition rates in anxious children were higher for the scream task in the previous study, as compared with attrition rates for anxious children in the current study. Yet, the sample size in the current study was small. Moreover, when the current study directly compared reported anxiety across tasks, the main effect of task was significant, with the scream eliciting more fear than the bells. Future studies with larger samples should further address the important question of comparing the tolerability of the two tasks. Nevertheless, based on data across the two studies, the bell task appears to be better tolerated than the scream task.

A basic premise in the fear-conditioning literature is that individual differences in fear conditioning and extinction are trait-like patterns of responding that show stability over time. However, to the best of our knowledge only three previous studies, all in adults, examined the temporal stability of these indices.[18-20] Results from the present study are generally in line with these previous studies indicating moderate stability over time. Interestingly, research implies that temporal stability is better when tested in two different sets of stimuli, as was done in the current study.[18] Future studies aiming to examine changes in fear conditioning and extinction over time, for example, before and after treatment, might consider using different stimuli, such as the stimuli from the two tasks used in the current study. However, as discussed in the next section, differences in the social and nonsocial nature of the stimuli should be considered.

FEAR CONDITIONING AND EXTINCTION IN ANXIOUS AND NONANXIOUS YOUTH AND ADULTS

The main effect of anxiety on self-reported fear found in the current study is similar to results in two previous studies that used the screaming lady task, in which anxious participants reported greater fear to both CSs than nonanxious participants. [1,5] Yet, in a pediatric study that used a different conditioning paradigm with geometric shapes, anxious children rated the valence of the CS+ more negatively than nonanxious children after conditioning and extinction, but no main effects of anxiety emerged for the CS− or for differential fear.[4] Therefore, the inconsistent results concerning extinction could have resulted in lower levels of reported fear to nonsocial (e.g., geometric shapes) than the social stimuli used in the screaming lady task, particularly among anxious participants. In addition, previous studies in anxious children[4,6] and adults[2] report a main effect of anxiety not only on self-reported fear but also on other psychophysiological indices such as SCR and FPS, effects not seen in the current study. In summary, general levels of heightened fear during fear conditioning and extinction are observed in anxious compared to nonanxious youth and adults.

Although perturbations in fear conditioning and extinction are thought to play a role in the etiology and maintenance of anxiety disorders, results in the current study indicate that both anxious and nonanxious individuals have comparable levels of differential conditioning and extinction, as measured by all three dependent variables. These results further support prior discriminative fear-conditioning studies in adults.[2] However, findings in the current study and other studies in pediatric anxiety disorders show similar effects in conditioning but different effects in extinction. That is, all of the pediatric studies, like the studies in adults, failed to find differences between anxious and nonanxious children in differential learning during fear conditioning. [1,4-6] Yet, the current findings differ from previous reports in which anxious children exhibit extinction-related deficits in SCR and FPS, as compared to nonanxious children.[4,6,7] In these studies, anxious children, but not healthy participants, showed larger SCR and FPS to CS+ than CS- after extinction, indicating resistance to extinction. Finally, based on self-reported fear both anxious and nonanx-ious participants were not extinguished. The lack of extinction, as indexed by self-report, was also evident in our first two studies with the scream task.[1,5] Moreover, although not widely discussed, prior studies repeatedly show that subjects' verbal reports regarding conditioning experiences do not reflect their autonomic responses, with patterns that appear quite similar to the patterns in our past two studies.[21-24]

Several methodological differences among studies may explain their differing results. Studies identifying extinction-related effects used nonsocial stimuli (geometric shapes or cartoon figures); whereas paradigms using social stimuli did not show these extinction-related effects using SCR. As discussed below, task-based differences were also found in the current study. Taken together, it appears that fear conditioning elicits comparable differential learning between anxious and nonanx- ious youth during fear conditioning. Nevertheless, differences in extinction appear to vary as a function of outcome measure, study paradigm, and sample characteristics.

TASK-SPECIFIC RESPONSES

Differences in stimuli could explain other discrepant findings across the two tasks obtained in the current study. For example, the difference in self-reported fear between the CS+ and CS− was greater in the bell task than in the screaming lady task, again, due to higher levels of fear elicited by the social CS− in the screaming lady task relative to the nonsocial CS− in the bell task. Similarly, anxious participants reported higher levels of fear to the social stimuli used in the screaming lady task compared to the nonsocial stimuli used in the bell task, even prior to the fear acquisition phase (preconditioning).

Differential SCR responses to the CS+ versus CS− in fear conditioning among adults were observed in the bell task, but unexpectedly, were lacking in the screaming lady task. This effect is surprising given that previous studies using this,[1,5] and similar tasks consistently report differential conditioning in SCR.[2] One possibility that may account, at least partially, for the lack of SCR- conditioning effects in adults involves the inclusion criteria applied in the current study. Some previous studies exclude SCR nonresponders;[2] however, all individuals, including responders and nonresponders, were included here, since these subjects provided useful data for EMG and self-reported fear. Indeed, the rate of SCR nonresponders was higher in adults (26.2%) than in youth (5.3%). Another possibility is age differences in SCR. Overall, levels of adult SCR were lower than in children. Such age-related differences in SCR (i.e., greater overall SCR response in youth than adults) have been reported in previous fear-conditioning studies.[1,5] Finally, this differential conditioning across the two tasks could relate to differences in physiological responses to the neutral CS used in the bell task (bell cartoons) compared to the screaming lady task (human face), with the latter eliciting higher levels of SCR to the CS−.

Along the same lines, the order in which the tasks were administered in the present study had no effect on SCR but did affect self-reported fear and FPS. The bell task elicited stronger differential learning effects, indexed by self-reported fear, in youth when the screaming lady task was delivered first. Conversely, in adults differential learning was stronger in the screaming lady task when the bell task was administered first. Moreover, anxious youth showed stronger differential effects in the bell task indexed by EMG when the screaming lady task was delivered first.

Strong differential conditioning effect depends on a potent and ecologically valid UCS paired with neutral stimuli. If indeed a screaming lady UCS is more potent than a bell UCS, stronger conditioning effects are expected to arise, particularly in adults who completed this task after the bell task. Yet, social stimuli are more salient to children and even more so to anxious children. Thus, the stimuli used in the bell task could have been perceived as more neutral than the faces in the screaming lady task, resulting in a stronger differential conditioning effect. This effect could have been exacerbated by the order of the tasks, as participants knew what to expect in the second task, which may have contributed to general levels of decreased fear.

LIMITATIONS

Although the present study compared anxious and nonanxious youth and adults on two different fear- conditioning tasks using three dependent variables, several limitations should be noted. First, the sample sizes were relatively small, particularly for the anxious youth. This may have limited our ability to observe between-group differences in fear conditioning and extinction. Small sample size also prohibited our ability to test for potential differences between anxiety subtypes, which may be relevant given differences in fear conditioning between anxiety subtypes in adults.[25,26] However, despite modest sample sizes, the study acquired a considerable amount of data from over 150 conditioning and extinction sessions. A second 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 or FPS. Although this approach allows findings to better represent the general anxiety population, it could reduce our ability to detect some between-group differences.

Other limitations relate to methodological constraints of data acquisition. First, we did not obtain trial-by-trial measurement of self-reported fear. Unlike SCR and FPS, self-report was collected only after participants completed the entire conditioning and extinction phases. A trial-by-trial measure can differ from pre- to postphase measure,[27] which is particularly relevant for subjective self-report, given that this measure was the only index that did not show extinction. Finally, because we used two different psychophysiological measurements during each trial (SCR and EMG), we could not analyze SCR in different time intervals as some previous studies have done.[4,6] Nonetheless, some data suggest that temporal stability of SCR is highest for the first interval response after stimulus onset.[18]

CONCLUSION

In summary, the present study found overall similar patterns of differential fear conditioning and extinction in anxious and nonanxious youth and adults using two different fear-conditioning tasks. These findings are consistent with prior pediatric anxiety studies that have reported similar between-group fear learning processes, but different from some of these studies pointing to differences in extinction.

Results from the current study indicate that the bell- conditioning task is both potent in eliciting fear responses and tolerable for anxious youth. Selection of one or another fear-conditioning task for future research should be considered in light of the research question as well as the clinical and age characteristics of the targeted population. Namely, stimuli in the scream task are social in nature, and the task could generate high rate of attrition in clinical and young populations due to the high levels of fear that the task may generate. The bell task, in contrast, consists of nonsocial, cartoon like, stimuli that generate lower levels of fear and may be better tolerated by young children. Finally, because the bell and the scream tasks share many similar features, it is important to compare data collected with each of the two tasks.

Extinction-based therapies (e.g., exposure therapy) representthe mainstay of anxiety disorder treatment. Because some patients fail to respond to these traditional Cognitive Behavioral Therapy (CBT) interventions, it may be important to have an index of individual differences in fear and extinction learning. The current report provides two such task options (i.e., the bell task and scream task), each with relative advantages and disadvantages. Therefore, using these tasks to understand impairments in fear conditioning and extinction and measuring them prior to treatment could prove meaningfully. This work eventually could lead to improvements in extinction-driven treatment by using pretreatment measures of conditioning and extinction to identifying individuals with particularly strong or weak response to these treatments. Similarly, such pretreatment measures also could provide markers to be targeted in research on novel treatments.

Acknowledgments

Contract grant sponsor: Intramural Research Program of the National Institute of Mental Health, National Institutes of Health; Contract grant sponsor: Marie Curie Career Integration Grant and Israel Science Foundation ISF-1377/14 (TS); Contract grant sponsor: National Institute of Mental Health R00-091183 (JCB).

REFERENCES

1. Britton JB, Grillon C, Lissek S, et al. Response to learned threat: an fMRI study in adolescent and adult anxiety. Am J Psychiatr 2013;170(10):1198–1204.
2. Lissek S, Powers AS, McClure EB, et al. Classical fear conditioning in the anxiety disorders: a meta-analysis. Behav Res Ther 2005;43(11):1391–424. [PubMed: 15885654]
3. Shechner T, Hong M, Britton JC, et al. Fear conditioning and extinction across development: evidence from human studies and animal models. Biol Psychol 2014;100C:1–12.
4. Craske MG, Waters AM, Lindsey Bergman R, et al. Is aversive learning a marker of risk for anxiety disorders in children? Behav Res Ther 2008;46(8):954–967. [PubMed: 18539262]
5. Lau JY, Lissek S, Nelson EE, et al. Fear conditioning in adolescents with anxiety disorders: results from a novel experimentalparadigm. J Am Acad Child Adolesc Psychiatr 2008;47(1):94–102.
6. Waters AM, Henry J, Neumann DL. Aversive Pavlovian conditioning in childhood anxiety disorders: impaired response inhibition and resistance to extinction. J Abnorm Psychol 2009;118(2): 311–321. [PubMed: 19413406]
7. Liberman LC, Lipp OV, Spence SH, March S. Evidence for retarded extinction of aversive learning in anxious children. Behav Res Ther 2006;44(10):1491–1502. [PubMed: 16360117]
8. Neumann DL, Waters AM, Westbury HR. The use of an unpleasant sound as the unconditional stimulus in aversive Pavlovian conditioning experiments that involve children and adolescent participants. Behav Res Methods 2008;40(2):622–625. [PubMed: 18522074]
9. Pine DS, Helfinstein SM, Bar-Haim Y, et al. Challenges in developing novel treatments for childhood disorders: lessons from research on anxiety. Neuropsychopharmacology 2009;34(1):213–228. [PubMed: 18754004]
10. Pattwell SS, Duhoux S, Hartley CA, et al. Altered fear learning across development in both mouse and human. Proc Natl Acad Sci USA 2012;109(40):16318–16323. [PubMed: 22988092]
11. Gao Y, Raine A, Venables PH, et al. The development of skin conductance fear conditioning in children from ages 3 to 8 years. Dev Sci 2010;13(1):201–212. [PubMed: 20121876]
12. Neumann DL, Waters AM, Westbury HR, Henry J. The use of an unpleasant sound unconditional stimulus in an aversive conditioning procedure with 8- to 11-year-old children. Biol Psychol 2008;79(3):337–342. [PubMed: 18822341]
13. Glenn CR, Klein DN, Lissek S, et al. The development of fear learning and generalization in 8-13 year-olds. Dev Psychobiol 2012;54(7):675–684. [PubMed: 22072276]
14. Lau JY, Britton JC, Nelson EE, et al. Distinct neural signatures of threat learning in adolescents and adults. Proc Natl Acad Sci USA 2011;108(11):4500–4505. [PubMed: 21368210]

15. Fluvoxamine for the treatment of anxiety disorders in children and adolescents. The Research Unit on Pediatric Psychopharmacology Anxiety Study Group. N Engl J Med 2001;344(17):1279–1285. [PubMed: 11323729]
16. Walkup JT, Albano AM, Piacentini J, et al. Cognitive behavioral therapy, sertraline, or a combination in childhood anxiety. N Engl J Med 2008;359(26):2753–2766. [PubMed: 18974308]
17. Prokasy WF, Ebel HC. Three components of classically conditioned Gsr in human subjects. J Exp Psychol 1967;73(2): 247–256.
18. Fredrikson M, Annas P, Georgiades A, et al. Internal consistency and temporal stability ofclassically-conditioned skin-conductance responses. Biol Psychol 1993;35(2):153–163. [PubMed: 8507744]
19. Torrents-Rodas D, FullanaMA, BonilloA, et al. Testing the temporal stability of individual differences in the acquisition and generalization of fear. Psychophysiology 2014;51(7):697–705. [PubMed: 24673651]
20. Zeidan MA, Lebron-Milad K, Thompson-Hollands J, et al. Test- retest reliability during fear acquisition and fear extinction in humans. CNS Neurosci Ther 2012;18(4):313–317. [PubMed: 21592319]
21. Hammond GS, Baer PE, Fuhrer MJ. Retention of differential autonomic conditioning and memory for conditional stimulus relationships. Psychophysiology 1980;17(4):356–362. [PubMed: 7394130]
22. Lissek S, Baas JM, Pine DS, et al. Airpuff startle probes: an efficacious and less aversive alternative to white-noise. Biol Psychol 2005;68(3):283–297. [PubMed: 15620795]
23. Sanderson RE, Campbell D, Laverty SG. Traumatically conditioned responses acquired during respiratory paralysis. Nature 1962;196:2135–2136. [PubMed: 13976228]
24. Schell AM, Dawson ME,Marinkovic K. Effects ofpotentiallypho- bic conditioned stimuli on retention, reconditioning, and extinction of the conditioned skin conductance response. Psychophysiology 1991;28(2):140–153. [PubMed: 1946880]
25. Michael T, Blechert J, Vriends N,et al. Fear conditioning in panic disorder: enhanced resistance to extinction. J Abnorm Psychol 2007;116(3):612–617. [PubMed: 17696717]
26. Blechert J, Michael T, Vriends N, et al. Fear conditioning in posttraumatic stress disorder: evidence for delayed extinction of autonomic, experiential, and behavioural responses. Behav Res Ther 2007;45(9):2019–2033. [PubMed: 17442266]
27. Lipp OV, Oughton N, LeLievre J. Evaluative learning in human Pavlovian conditioning: extinct, but still there? Learn Motiv 2003;34(3):219–239.

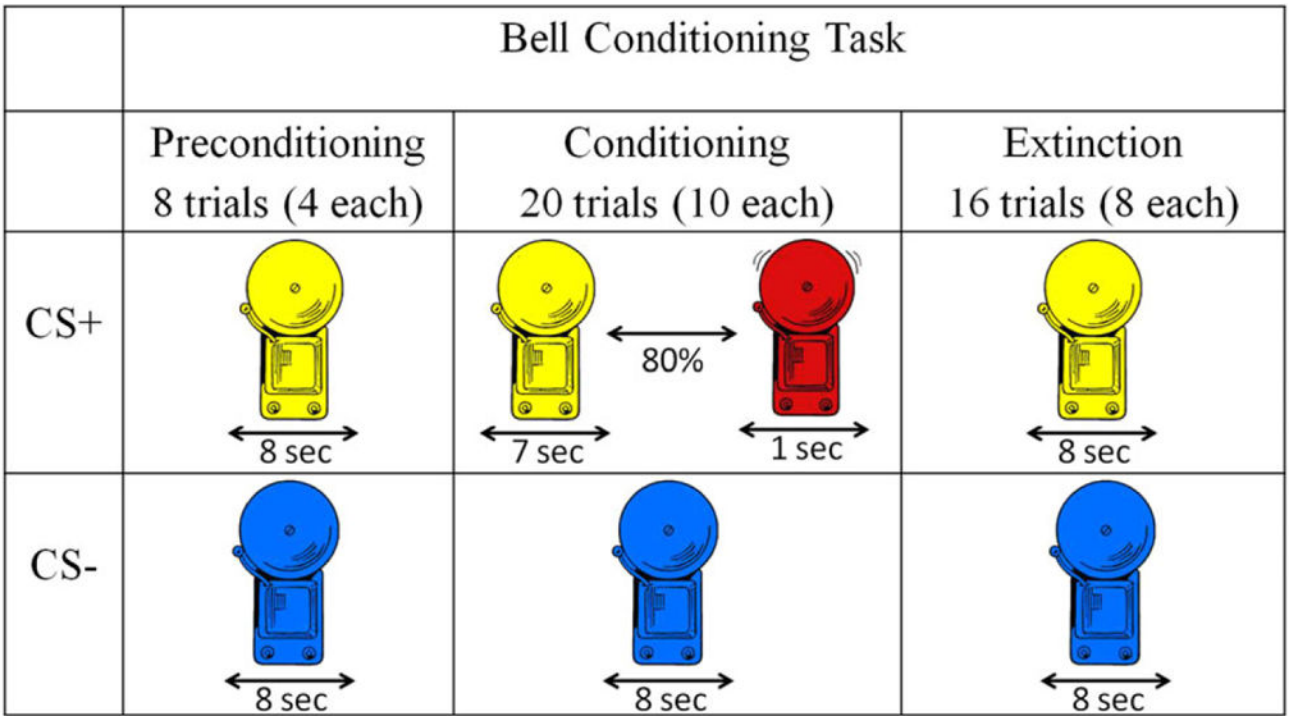

Figure 1.
Bell-conditioning task

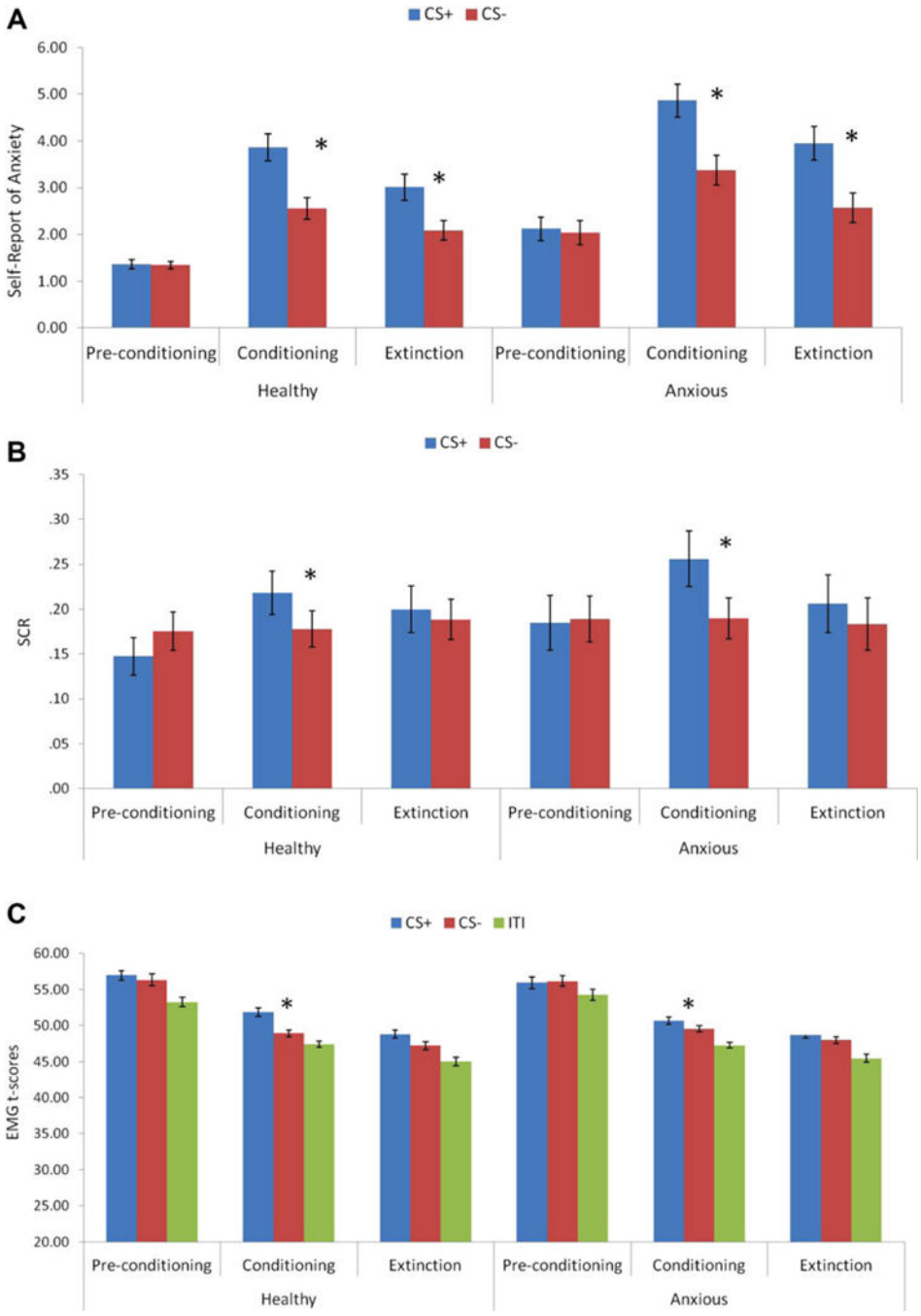

Figure 2.

Fear conditioning and extinction in anxious and nonanxious participants collapsed across age and tasks.

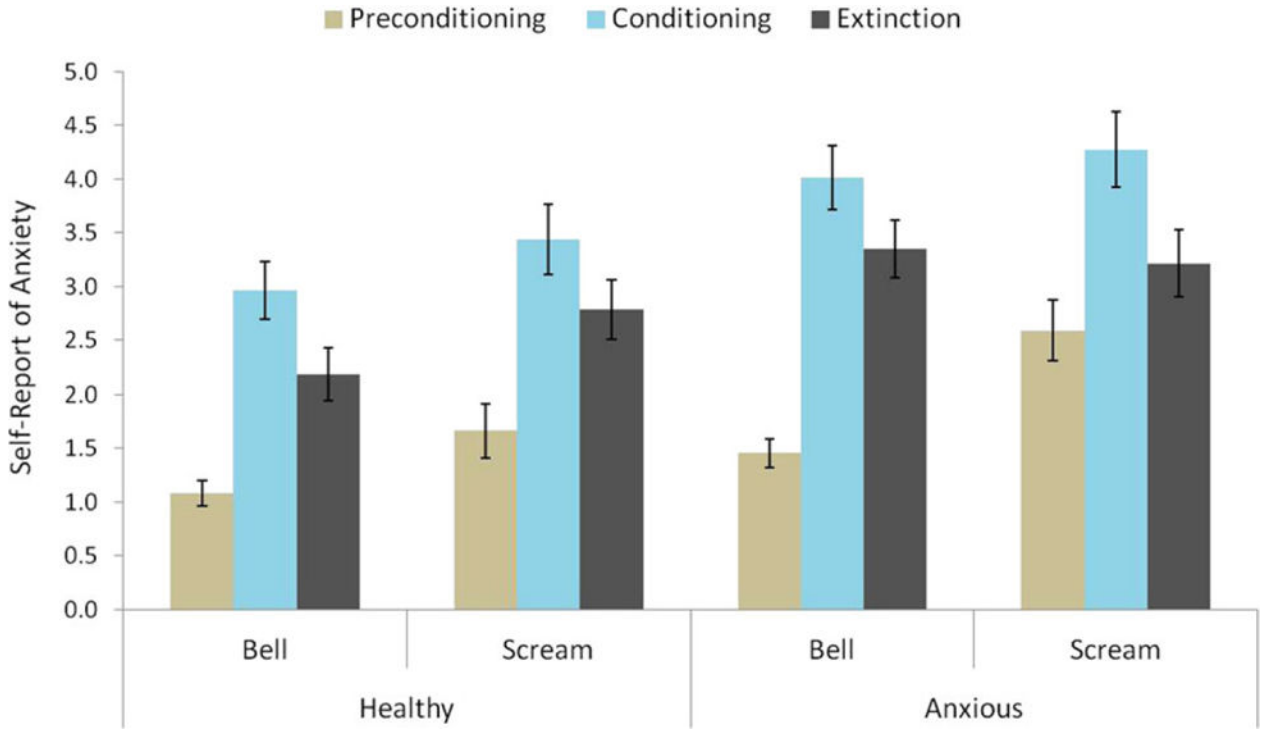

Figure 3.
The effect of task, phase, and anxiety group on self-reported fear.

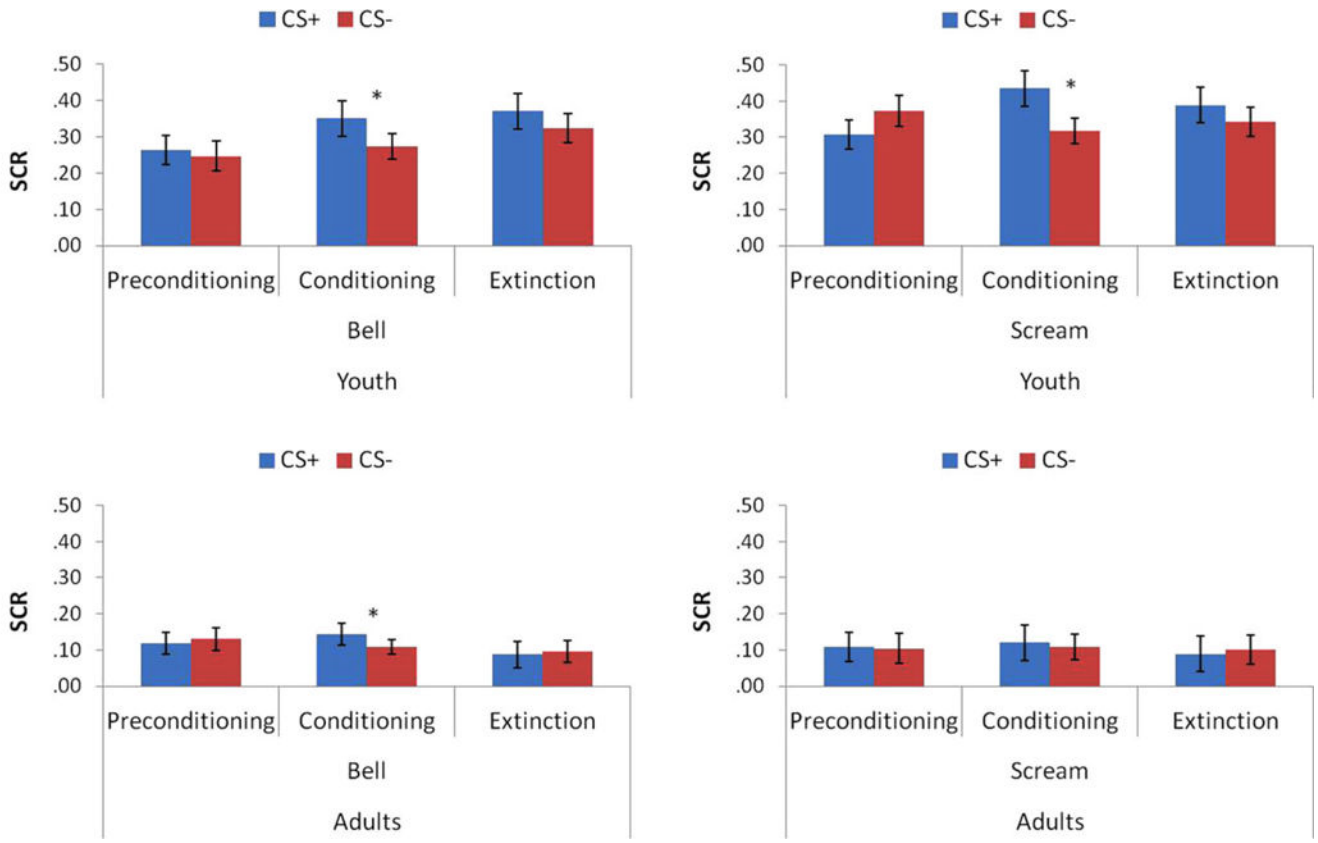

Figure 4.
The effect of task, phase, stimuli, and age group on SCR

TABLE 1.

Sample demographics

	Patients		Healthy	
Whole sample (*n* = 84)	**Youths (*n* = 15)**	**Adults (*n* = 22)**	**Youths (*n* = 17)**	**Adults (*n* = 30)**
Subject anxiety diagnosis				
GAD	1	6		
SoPh	4	4		
SAD	1			
GAD and SoPh	2	9		
GAD and SAD	0			
GAD and PD	0	3		
GAD and SpecPh	2			
SoPh and SAD	0			
GAD, SoPh, and SAD	3			
GAD, SoPh, and SpecPh	2			
Anxiety symptom scores				
Self-reported SCARED total	26.9 (14.9)		7.7 (5.6)	
Parent-reported SCARED total	34.7 (14.4)		4.8 (5.4)	
Self-reported LSAS total		58.5 (25.1)		23.2 (20.2)
Demographics				
Mean age	11.5(2.57)	29.2(8.35)	13.0 (2.97)	29.7 (7.12)
No. of females (percentage of sample)	7 (46.7)	17(77.3)	9 (52.9)	11 (36.7)
Two WASI subscales IQ	116.5(13.6)	114.9 (11.2)	110.3(8.3)	116.6(12.8)

Note: GAD, generalized anxiety disorder; SoPh, social phobia; SAD, separation anxiety; PD, panic disorder; SpecPh, specific phobia; SCARED, Self-Report for Childhood Anxiety Related EmotionalDisorder; LSAS, Liebowitz social anxiety scale; PARS, Pediatric Anxiety Rating Scale;WASI, Wechsler Abbreviated Scale of Intelligence.

Two WASI subscales are vocabulary and matrix reasoning.

TABLE 2.

Interclass correlations (ICC) coefficients [confidence intervals] between the three dependent variables across the two tasks

			Bell-conditioning task			
			Conditioning		Extinction	
			CS+	CS-	CS+	CS-
Scream-conditioning task	Self-report	Conditioning	**.62** [**] **[.41–.75]**	**.45** [*] **[.16–.64]**		
		Extinction		.	**.54** [**] **[.30–.70]**	**.42** [*] **[.11–.62]**
	SCR	Conditioning	**.65** [**] **[.46–.78]**	**.63** [**] **[.42–.76]**		
		Extinction		.	**.48** [*] **[.19–.66]**	**.46** [*] **[.16–.65]**
	EMG	Conditioning	**.36** [*] **[.01–.59]**	**.34** [*] **[−.20–.56]**		
		Extinction			−.02 [−.59–.34]	.26 [−.14–.52]

[**] Note: $P < .001$;

[*] $P < .05$. Significant correlations are indicated in bold.

TABLE 3.

ANOVA results for Phase × Stimuli × Anxiety × Age for the three dependent variables

	Self-report			SCR			EMG		
Effect	***df***	***F***	**Significant**	***df***	***F***	**Significant**	***df***	***F***	**Significant**
Effects of conditioning									
Phase	2, 152	**71.32****	*P*< .001	2, 146	1.92	*P*= .151	2, 148	**105.33****	*P*< .001
Stimuli	1, 76	**49.45****	*P*< .001	1,73	**8.17***	*P*= .006	2, 148	**66.87****	*P*< .001
Phase × Stimuli	2, 152	**21.38****	*P*< .001	2, 146	**8.48***	*P*< .001	4, 296	**4.36***	*P*= .002
Effects of age									
Age	1, 76	0.24	*P*= .628	1,73	**24.28***	*P*< .001	1, 74	**4.89***	*P*= .030
Phase × Age	2, 152	0.54	*P*= .582	2, 146	**4.69***	*P*= .011	2, 148	2.50	*P*= .085
Stimuli × Age	1, 76	0.00	*P*= .985	1,73	**5.95***	*P*= .017	2, 148	0.36	*P*= .699
Phase × Stimuli × Age	2, 152	0.20	*P*= .820	2, 146	**3.84***	*P*= .024	4, 296	0.58	*P*= .676
Effects of anxiety									
Anxiety	1,76	**10.11***	*P*= .002	1,73	0.43	*P*= .515	1, 74	0.18	*P*= .671
Phase × Anxiety	2, 152	0.40	*P*= .673	2, 146	0.08	*P*= .973	2, 148	0.28	*P*= .757
Stimuli × Anxiety	1,76	1.46	*P*= .231	1,73	1.56	*P*= .217	2, 148	1.68	*P*= .190
Phase × Stimuli × Anxiety	2, 152	0.56	*P*= .571	2, 146	0.70	*P*= .501	4, 296	0.46	*P*= .764
Phase × Stimuli × Anxiety × Age	2, 152	1.06	*P*= .350	2, 146	1.37	*P*= .256	4, 296	0.72	*P*= .583

Note: SCR,skin conductance response; EMG, electromyography; *df,* degrees of freedom. Significant effects are indicated in bold.