



## Comparing electric shock and a fearful screaming face as unconditioned stimuli for fear learning

Catherine R. Glenn<sup>a,\*</sup>, Lynne Lieberman<sup>b</sup>, Greg Hajcak<sup>c</sup>

<sup>a</sup> Department of Psychology, Harvard University, Cambridge, MA, 02138, United States

<sup>b</sup> Section on Neurobiology of Fear and Anxiety, National Institute of Mental Health, Bethesda, MD 20892, United States

<sup>c</sup> Department of Psychology, Stony Brook University, Stony Brook, NY 11794, United States

### ARTICLE INFO

#### Article history:

Received 14 April 2012

Received in revised form 7 September 2012

Accepted 14 September 2012

Available online 21 September 2012

#### Keywords:

Fear learning

Fear conditioning

Fear-potentiated startle

Startle reflex

Unconditioned stimuli

### ABSTRACT

The potency of an unconditioned stimulus (UCS) can impact the degree of fear learning. One of the most common and effective UCSs is an electric shock, which is inappropriate for certain populations (e.g., children). To address this need, a novel fear learning paradigm was recently developed that uses a fearful female face and scream as the UCS. The present study directly compared the efficacy of the screaming female UCS and a traditional shock UCS in two fear learning paradigms. Thirty-six young adults completed two fear learning tasks and a measure of trait anxiety; fear learning was indexed with fear-potentiated startle (FPS) and self-reported fear ratings. Results indicated comparable FPS across the two tasks. However, larger overall startle responses were exhibited in the shock task, and participants rated the shock UCS and overall task as more aversive than the screaming female. In addition, trait anxiety was only related to FPS in the fear learning task that employed a shock as the UCS. Taken together, results indicate that, although both UCS paradigms can be used for fear conditioning (i.e., to produce differences between CS+ and CS−), the shock UCS paradigm is more aversive and potentially more sensitive to individual differences in anxiety.

© 2012 Elsevier B.V. All rights reserved.

### 1. Introduction

Fear learning refers to the process by which a neutral stimulus (or CS+) produces a fearful response after being paired with an aversive unconditioned stimulus (UCS). Following fear conditioning, organisms exhibit heightened fear responding when presented with a CS+ (the stimulus that predicts impending threat, or UCS), relative to the CS− (a stimulus that specifically signals safety, or absence of UCS; Davis et al., 1993). Previous studies have consistently used fear-potentiated startle (FPS) to measure fear learning because the magnitude of the eye-blink startle reflex is reliably augmented during fearful states (Hamm et al., 1993; Lang et al., 1990). For instance, in a fear learning paradigm with paired and unpaired conditions, individuals exhibited a heightened startle response (i.e., FPS) during a blue light (the CS+) that had been repeatedly paired with an electric shock (UCS), whereas those in the unpaired condition did not exhibit FPS during the blue light because it did not predict the UCS (Grillon and Davis, 1997).

Notably, the nature of the UCS itself impacts the degree of fear learning. That is, more potent UCSs are related to greater baseline levels of arousal (Grillon et al., 2004), faster fear acquisition (Cook et al., 1986),

greater magnitude of the fear response (Cook et al., 1986; Grillon et al., 2004), as well as increased resistance to fear extinction (Cook et al., 1986). Fear conditioning is most successful when the UCS is both potent and survival-relevant (Britton et al., 2010). Thus, a weak UCS may not elicit significant differences in fear learning either within (i.e., between CS+ and CS−) or between individuals (e.g., anxious vs. and non-anxious groups; Britton et al., 2010).

An electric shock is one of the most widely used UCSs in fear learning studies in both human and non-human animals (Brown et al., 1951; Davis et al., 1993; Grillon, 2002). However, a shock may not be a feasible UCS for use with certain populations, such as children and adolescents (referred to collectively as *children*). Instead, alternative, and in some cases milder UCSs, have been used in fear learning paradigms with children, including a loud tone (Craske et al., 2008; Liberman et al., 2006), metal scraping on a slate (Neumann et al., 2008; Waters et al., 2009), or an air blast to the larynx (Borelli et al., 2011; Grillon et al., 1998; Reeb-Sutherland et al., 2009). However, the magnitude of fear learning using these shock-alternative UCSs has been variable across studies.

In an effort to create an appropriate and effective fear conditioning paradigm for children, Lissek et al. (2005a) developed a novel UCS — a fearful female face paired with a shrieking female scream (also see Grillon et al., 2008; Lau et al., 2008). This novel and survival-relevant UCS has successfully elicited greater fear responses during CS+ relative to CS− when indexed by subjective fear ratings (Lau et al., 2008) and startle responses (Glenn et al., 2012; Schmitz et al., 2011). Although the screaming female UCS paradigm has been found to be comparable

\* Corresponding author at: Harvard University, William James Hall, 1280, 33 Kirkland Street, Cambridge, MA 02138, United States. Tel.: +1 617 496 8563; fax: +1 617 496 9462.

E-mail addresses: [catherineglenn@fas.harvard.edu](mailto:catherineglenn@fas.harvard.edu) (C.R. Glenn), [lnlieberman@gmail.com](mailto:lnlieberman@gmail.com) (L. Lieberman), [greg.hajcak@stonybrook.edu](mailto:greg.hajcak@stonybrook.edu) (G. Hajcak).

to an alarm, a loud tone, and white noise, as measured by subjective ratings of fear (Britton et al., 2010), previous studies have not examined how this novel UCS compares to the potency of an electric shock. In addition, no studies have compared the screaming female UCS to other UCSs using fear-potentiated startle – one of the most reliable physiological measures of fear learning (Lang et al., 1990).

Therefore, the main purpose of the current study was to examine the efficacy of the screaming female UCS paradigm compared to the more commonly used electric shock UCS. Although an electric shock is a more noxious UCS, the screaming female UCS may have its own unique strengths. In particular, the screaming female may be a more survival-relevant UCS than an electric shock. Survival relevance refers to the likelihood of a particular stimulus to have served as a consistent threat throughout history (Ohman and Mineka, 2001); for instance, a snake would be considered a more survival-relevant stimulus than a flower. Moreover, stimuli that are relatively newer threats, such as weapons (or in this case, electric shocks), may not elicit the same magnitude of fear response compared to more longstanding threats to survival, such as predatory animals (or in this case, social cues that signal threat in the environment; see reviews: Ohman, 1986; Ohman and Mineka, 2001; Seligman, 1971). Given that the two UCSs may be advantageous for different reasons, we hypothesized that participants would exhibit similar fear learning patterns (i.e., differences between CS+ and CS– startle magnitudes) when either UCS paradigm was utilized. In addition, we expected self-reported ratings of fear to be higher for CS+ than CS– in both tasks, but not significantly different between the two paradigms.

Notably, a large and growing literature has linked dysfunction in fear learning to the development and maintenance of anxiety disorders in adults (Britton et al., 2010; Lissek et al., 2005b; Mineka and Oehlberg, 2008). A recent meta-analysis of fear learning studies with adult anxiety patients indicates that the link between anxiety and fear learning is complex (Lissek et al., 2005b). In simple fear conditioning studies where only a threat cue (CS+) is presented, anxiety is related to greater fear acquisition. However, in discrimination paradigms where both threat and safety cues are presented (CS+ compared to CS–), anxiety is related to overall larger startle magnitudes but not to greater fear learning. Given the association between anxiety and fear acquisition in simple conditioning designs, Lissek et al. (2005b) suggested that the lack of fear conditioning differences in discrimination paradigms may be due to elevated responses during safety cues (CS–), and thus may indicate generalization of the fear response. Although much less is known about the pathophysiology of anxiety disorders in children, fear learning studies in this population indicate that the associations between anxiety and fear learning may be similar to those found in adults. That is, previous studies have found that children with anxiety disorders (Lau et al., 2008) and adolescent females at high-risk for anxiety disorders (Grillon et al., 1998) exhibit elevated startle magnitudes across all stimuli, rather than greater fear acquisition specifically.

Given the increasing use of the screaming female UCS paradigm to examine fear learning in children (e.g., Glenn et al., 2012; Lau et al., 2008; Schmitz et al., 2011), it is important not only to examine how the UCS paradigm compares to an electric shock UCS, but also to assess whether it is sensitive to individual differences in anxiety. Therefore, a secondary goal of the present study was to examine how FPS in the two UCS paradigms related to trait anxiety. Based on existing research in adults and children, we predicted that trait anxiety would be related to greater overall startle magnitudes across the two tasks.

## 2. Materials and methods

### 2.1. Participants

The original sample consisted of 47 young adults recruited from a college population. Eleven participants were excluded from analyses for the following reasons: (a) stopping the startle task before it concluded

( $n = 1$ ), (b) failing to exhibit any measurable startle response on 2/3 of trials ( $n = 3$ ), (c) excessive artifacts during the baseline period (the 50 ms period before the onset of the startle probe;  $n = 5$ ), and (d) outliers in the startle data (i.e., startle magnitudes more than 2 standard deviations above or below the mean;  $n = 2$ ).

The final sample included 36 participants (25 female). The average age of the sample was 19.44 ( $SD = 1.89$ ), and the largest ethnic groups were Caucasian (41.7%), Asian (27.8%), and Hispanic (16.7%). Participants excluded from the analyses were not significantly different from participants included in the analyses based on age ( $t[45] = 0.71$ ,  $p = .481$ ) or gender ( $\chi^2[1, N = 47] = 0.83$ ,  $p = .361$ ). However, there were ethnicity differences between the two groups: included participants were less likely to be African American ( $\chi^2[1, N = 47] = 6.49$ ,  $p = .011$ ) and more likely to be Caucasian ( $\chi^2[1, N = 47] = 3.98$ ,  $p = .046$ ) than participants excluded from the analyses.

### 2.2. Stimuli and presentation

#### 2.2.1. Faces task

The fear learning stimuli used in this task were based on Lau et al. (2008). The task described below was recently used to measure fear learning in 8 to 13 year-olds (Glenn et al., 2012). In this task, the CS+ and CS– were two neutral female faces (NimStim: 01F, 03F; Tottenham et al., 2009); CSs were counterbalanced across participants. The UCS was a fearful female face (same actress as the neutral CS+) paired with a loud female scream. The UCS scream was presented over computer speakers and was experienced by the participant (at the ear while wearing headphones) at approximately 80 dB for 1 s. On 75% of CS+ trials, the CS+ was reinforced with the UCS; on these trials, the neutral CS+ face was presented for six seconds, and then replaced by the fearful female face for an additional three seconds, and this switch was accompanied by the loud female scream.

#### 2.2.2. Shapes task

In this task, the CS+ and CS– were a square and a circle; again, CSs were counterbalanced across participants. The UCS was a shock delivered to the participant's left tricep with an electric stimulator (Contact Precision Instruments) that presented 60 Hz constant AC stimulation between 0 and 5 mA for 500 ms. The level of shock was chosen by each participant to be a level that was highly uncomfortable, but still tolerable (see Procedure for details). CS+ trials were reinforced with the UCS, immediately after the offset of the CS+, on 75% of CS+ trials.

#### 2.2.3. Faces and shapes tasks

There were eight CS+ and eight CS– trials, and CS– trials were never followed by the UCS. CSs were presented for six seconds and intertrial intervals (ITIs; CS offset to CS onset) ranged from 10 to 12 s. For each task, three pseudorandom trial orders were constructed and randomized between participants. The startle reflex was elicited with auditory startle probes – 50 ms, 105 dB bursts of white noise with instantaneous rise/fall time – which were presented binaurally through headphones using a noise/tone generator (Contact Precision Instruments; Cambridge, MA). Startle probes were presented during six of the eight CS+ and six of the eight CS– trials, 3.5–4.5 s after picture onset. However, CS+ reinforced trials and startle trials were not always the same; that is, the presence of a startle probe did not predict the UCS. In addition, startle probes were presented during four ITIs in each task.

Visual stimuli were presented with Presentation software (Neurobehavioral Systems, Inc; Albany, CA) on a 19-inch monitor 23 in. from the participant. Stimuli occupied 4 in. vertically and 2.5 in. horizontally of the computer screen. In the Faces task, visual stimuli were presented in gray scale; in the Shapes task, visual stimuli were presented in royal blue against a black background. Speakers, delivering

the UCS scream in the Faces task, were positioned approximately 25 in. from the participant.

#### 2.2.4. Trait anxiety

Trait anxiety was measured with the 20-item trait scale of the State-Trait Anxiety Inventory (STAI; Spielberger et al., 1970). Participants were instructed to indicate how much each statement described how they *generally* feel on a scale from 1 = *almost never* to 4 = *almost always*; the total scale ranges from 20 to 80. The STAI trait scale has demonstrated good reliability and validity in previous research (Sesti, 2000).

#### 2.3. Procedure

The project was approved by Stony Brook University's Institutional Review Board. Following informed consent, all participants completed both the Faces and Shapes tasks in a sound-attenuated chamber. Startle electrodes were attached first for all participants, regardless of task order.

Prior to beginning the Shapes task, participants chose their level of shock. The initial shock administered was mild and was increased in small increments until the participant selected a level of shock that was “*highly uncomfortable and that you're not looking forward to it, but that is still within your tolerance for pain.*” In the process of choosing their level of shock, participants received between one and ten shocks before the task began (the specific number of shocks each participant received was not recorded). However, the specific shock level that participants chose to be used in the current study was received only once during this calibration procedure. Next, participants completed a habituation phase, during which four non CS +/- shapes were presented along with four startle probes via headphones. Participants were told to ignore the startle probes. Instructions for the task were, “*In the next part of this experiment, you'll be viewing different shapes on the computer screen. Remember you chose a level of shock; in this part of the experiment, you will receive some shocks. If you pay attention to the shapes, you may learn to predict when the shock happens.*”

For those participants who completed the Shapes task first, shock electrodes were removed before beginning the Faces task. Before the Faces task began, participants were presented with the UCS scream to match the experience in the Shapes task (where the UCS was presented during shock selection prior to the start of the task). Participants then completed a habituation phase, during which four non CS +/- female faces were presented along with four startle probes. Participants were told to ignore the startle probes. Following the habituation phase, participants were told, “*In the next part of this experiment, you will see pictures of two women on the screen. While you are watching the pictures, one of the women may change to look scared and you will hear the scream through the speakers. If you pay attention, you may be able to predict when the scream happens.*”

After completing each fear learning task, participants were asked to identify which CS was followed by the UCS (i.e., which shape was followed by the shock and which face was followed by the screaming fearful face). In addition, participants were asked to rate how distressed/anxious they were while viewing the CSs (faces or shapes) on a scale from 0 = *not at all* to 3 = *very distressed/anxious*. Specifically, participants were asked, “*to what extent did you think 'uh-oh' or 'oh no' when you saw this (shape or face)?*” After both tasks were completed, participants were asked to select which UCS (scream, shock, or both equally), as well as which task (Faces, Shapes, or both equally) they found more distressing.

The order of fear learning tasks was counterbalanced across participants. The two tasks were completed back-to-back so only a few minutes elapsed between tasks. The procedures for the Faces and Shapes tasks were the same regardless of task order. Finally, participants completed the STAI trait scale. After study completion, participants were debriefed and compensated with course credit.

#### 2.4. Physiological data collection and analysis

Startle-elicited EMG activity was recorded using a PSYLAB Stand Alone Monitor Unit and BioAmplifier. Two Ag–AgCl electrodes were placed approximately 25 mm apart over the orbicularis oculi muscle beneath the left eye, with a third electrode placed in the center of the forehead as a ground. EMG activity was sampled at 1000 Hz, band-pass filtered between 30 and 500 Hz, and rectified in a window 200 ms wide window (beginning 50 ms before the onset of the startle probe). A 6-point running average was then applied to the rectified data to smooth out sharp peaks. The startle response in each trial was defined as the difference between the average activity in the 50 ms prior to the probe and the peak of the activity in the 150 ms after the probe.

For all participants, EMG activity was examined on a trial-by-trial basis. Three percent of trials in the Faces task and 4% of trials in the Shapes task were rejected due to excessively noisy baselines. Less than 1% of acceptable startle trials in the Faces and Shapes tasks showed no clearly defined startle response and therefore were considered non-responses and scored as zeroes. Startle responses were examined both in terms of raw data, and were also converted to T-scores to reduce between-subject variability.

#### 2.5. Data analytic plan

Fear learning in the two tasks was statistically evaluated through repeated measures ANOVA with the Greenhouse–Geisser correction applied. To examine the impact of completing the Shapes or Faces task first, task order was included in all analyses as a between-subjects factor. Self-reported fear ratings were examined using a 2 (task order: Faces first, Shapes first)  $\times$  2 (task: Faces, Shapes)  $\times$  2 (CS: CS+, CS–) mixed model repeated measures ANOVA. All significant interactions and main effects were followed by post hoc comparisons as appropriate.

Next, startle responses were examined using a 2 (task order: Faces first, Shapes first)  $\times$  2 (task: Faces, Shapes)  $\times$  3 (stimulus: CS+, CS–, ITI) mixed model repeated measures ANOVA. All significant interactions and main effects were followed by post hoc comparisons as appropriate. Finally, bivariate correlations were used to examine: (1) associations between fear ratings and FPS in the two fear learning tasks, (2) the convergence between self-report and startle measures of fear learning, and (3) associations between fear measures and trait anxiety.

### 3. Results

#### 3.1. Self-report ratings

Fear ratings of the CS+ and CS– in the Shapes and Faces tasks are presented in Table 1. There was a significant task (Shapes, Faces)  $\times$  stimulus (CS+, CS–) interaction,  $F(1, 34) = 4.44$ ,  $p = .043$ . Follow-up analyses indicated that fear ratings for the CS+ were significantly higher than for the CS– in both the Shapes and Faces tasks ( $ps < .001$ ). In addition, the CS+ in the Shapes task was rated as more distressing/anxiety-provoking than the CS+ in the Faces task ( $p = .005$ ). However, there was no difference in ratings between the CS– in the Shapes and Faces tasks ( $p = .865$ ). None of the two- and three-way interactions including task order were significant (all  $ps > .19$ ).

In addition to rating the CS+ and CS–, participants were asked to report which UCS (shock, scream, or both equally) and which fear learning task (Shapes, Faces, or both equally) were more distressing. For the more distressing UCS, 53% of participants chose the electric shock, 30% chose the fearful scream, and 17% reported that both were equally distressing. Similarly, for the more distressing fear learning task question, 44% chose the Shapes task, 36% chose the Faces task, and 20% reported that both tasks were equally distressing. These ratings

**Table 1**  
Self-report fear ratings of conditioned stimuli<sup>1</sup> in the Shapes and Faces tasks.

	Shapes task		Faces task		t-test <sup>2</sup>
	M (SD)		M (SD)		
CS+	2.11 (0.71) <sup>a</sup>		1.60 (0.99) <sup>c</sup>		$t_{a,c} = 3.10^{**}$ ; $d = 1.34$
CS-	0.75 (0.84) <sup>b</sup>		0.72 (0.81) <sup>d</sup>		$t_{b,d} = 0.17^{\#}$ ; $d = 0.08$
t-test <sup>2</sup>	$t_{a,b} = 8.02^{***}$ ; $d = 3.92$		$t_{c,d} = 6.01^{***}$ ; $d = 2.19$		

<sup>1</sup>Self-report ratings of anxiety/distress while viewing CS+ and CS- on a scale from 0 = not at all to 3 = very distressed/anxious. The UCS in the Shapes task was an electric shock and the UCS in the Faces task was a fearful face paired with a female scream.

<sup>2</sup>Subscripts correspond to the CSs included in the paired-samples t-tests ( $df = 35$ ). Effect sizes expressed with Cohen's  $d$ .

<sup>#</sup>  $p < .05$ .  
<sup>\*\*</sup>  $p < .01$ .  
<sup>\*\*\*</sup>  $p < .001$ .

**Table 2**  
Startle magnitude means ( $\mu V$ ) and standard errors of means for the first half and second half of the Shapes and Faces fear learning tasks.

	Shapes task				Faces task			
	1st half		2nd half		1st half		2nd half	
	M	SEM	M	SEM	M	SEM	M	SEM
CS+	69.05	4.08	62.37	4.43	66.73	3.67	58.18	4.30
CS-	62.94	4.58	59.53	4.60	64.05	4.03	52.95	4.58
ITI	62.51	4.68	54.81	5.00	59.00	5.01	55.04	4.73

did not significantly differ as a function of task order ( $ps > .55$ ). The average score on the STAI was 42.53 ( $SD = 7.27$ , Range: 31–62).

### 3.2. Startle response

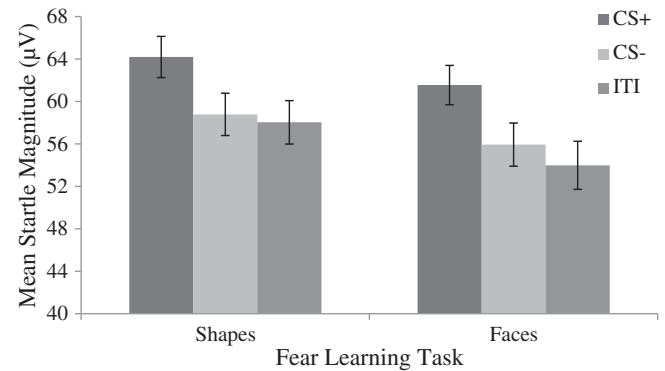
Raw startle means and SEMs for the first half and second half of the two fear learning tasks are presented in Table 2<sup>1</sup> and overall startle magnitudes for each task are displayed in Fig. 1. There was a significant task order (Faces first, Shapes first)  $\times$  task (Faces, Shapes) interaction,  $F(1, 34) = 6.92$ ,  $p = .013$ . Follow-up analyses indicated that when the Shapes task was completed first, startle magnitudes were significantly higher for the Shapes task ( $M = 65.19$ ,  $SD = 5.46$ ) compared to the Faces task ( $M = 58.26$ ,  $SD = 5.91$ ),  $t(16) = 4.51$ ,  $p < .001$ . However, there was no difference in the overall startle magnitudes for the two tasks when the Faces task was completed first (Faces:  $M = 56.18$ ,  $SD = 5.54$ ; Shapes:  $M = 56.01$ ,  $SD = 5.51$ ),  $t(18) = .08$ ,  $p = .938$ . In addition, there was a significant main effect of stimulus,  $F(2, 70) = 21.72$ ,  $p < .001$ , with larger responses during CS+ compared to both CS-,  $t(35) = 5.10$ ,  $p < .001$ , and ITI,  $t(35) = 5.94$ ,  $p < .001$ , across both tasks; however, there were no differences between CS- and ITI startle magnitudes,  $t(35) = 1.26$ ,  $p = .215$ . All remaining two- and three-way interactions were not significant (all  $ps > .75$ )<sup>2</sup>.

### 3.3. Convergence between self-report and startle measures

Difference scores of self-reported fear ratings (i.e., difference in fear ratings between CS+ and CS-) were uncorrelated across tasks,  $r(36) = -.01$ ,  $p = .944$ , regardless of task order ( $ps > .30$ ). Self-reported fear ratings in the Shapes task were related, at a trend level, to FPS during the Shapes task,  $r(36) = .32$ ,  $p = .060$ . However, there was

<sup>1</sup> We examined differences in fear conditioning in the two tasks by splitting each task into the first half and second half of trials. The task (Faces, Shapes)  $\times$  task half (first half, second half)  $\times$  stimulus (CS+, CS-, ITI) interaction was not significant,  $F(2, 50) = 0.89$ ,  $p = .415$ , nor were either of the two-way interactions including task half ( $ps > .70$ ). However, there was a main effect of task half with overall startle responses decreasing over the course of both tasks ( $F(1, 25) = 29.78$ ,  $p < .001$ ).

<sup>2</sup> Patterns of results were similar for both raw and T-scored data; therefore, only raw data are presented in the Results section.



**Fig. 1.** Raw startle magnitudes ( $\mu V$ ) during the Shapes and Faces tasks. Error bars indicate standard error of means.

no association between self-reported fear ratings and FPS in the Faces task,  $r(36) = .17$ ,  $p = .318$ . In addition, there were no associations between ITI startle magnitudes and self-reported fear in either the Shapes task,  $r(36) = -.29$ ,  $p = .085$ , or the Faces task,  $r(36) = .16$ ,  $p = .360$ .

In the overall sample, the magnitude of FPS in the Faces task was uncorrelated with FPS in the Shapes task,  $r(36) = .06$ ,  $p = .743$ . However, this association was moderated by task order. For individuals who completed the Shapes task first, FPS in the Shapes and Faces tasks were significantly correlated,  $r(17) = .50$ ,  $p = .041$ ; however, for participants who completed the Faces task first, FPS in the two tasks were uncorrelated,  $r(19) = -.21$ ,  $p = .384$ . These correlations were significantly different from each other ( $z = 2.08$ ,  $p = .037$ ).

In regard to anxiety, FPS during the Shapes task correlated significantly with STAI trait anxiety scores,  $r(36) = .36$ ,  $p = .031$ , whereas FPS during the Faces task was not significantly related to STAI trait anxiety,  $r(36) = -.12$ ,  $p = .503$ . These correlations were significantly different from each other ( $z = 2.11$ ,  $p = .035$ ). ITI startle magnitudes during the Shapes and Faces tasks were not significantly related to trait anxiety ( $r(36) = .09$ ,  $p = .604$ , and  $r(36) = -.15$ ,  $p = .387$ , respectively). Finally, there were no associations between trait anxiety and self-reported fear ratings in either the Shapes or Faces tasks ( $r(36) = .03$ ,  $p = .873$  and  $r(36) = .05$ ,  $p = .767$ , respectively). There were no significant differences in these associations based on task order (all  $ps > .15$ ).

## 4. Discussion

The purpose of the current study was to compare two UCSs for fear learning. Specifically, we examined whether a novel UCS developed for use with children – a fearful female face and scream (Lissek et al., 2005a) – was as effective in eliciting fear-potentiated startle (FPS) and increased ratings of fear as a UCS more commonly used with adults – an electric shock. In addition, the current study also examined associations between trait anxiety and FPS elicited by both the screaming female UCS and electric shock UCS.

In the present study, participants exhibited similar FPS patterns (i.e., larger startle magnitudes during CS+ compared to during CS-) across both discrimination paradigms, thereby indicating that the screaming female UCS was as effective as an electric shock for fear learning. In addition, consistent with the startle results, participants rated the CS+ as more fear-provoking than the CS- in both tasks.

However, findings from the current study indicate that, although FPS was observed in both fear learning paradigms, the shock UCS paradigm was more aversive and more sensitive to individual differences in anxiety. First, although the CS+ was rated as more aversive than the CS- in both tasks, the shock UCS and shock task were rated as significantly more aversive than the screaming female UCS and task. In addition, self-reported fear ratings were related to FPS during the shock task, at a trend level, but these measures were unrelated in the screaming female task. These findings suggest that there may be

greater convergence between subjective and physiological measures of fear when an electric shock is the UCS, but not when other less aversive stimuli are utilized. Further, FPS in the shock task was associated with trait anxiety, whereas FPS in the scream task was not. These results suggest that the two fear learning paradigms may not be assessing the same construct of fear, and that the screaming female paradigm may not elicit FPS that is sensitive to individual differences in trait anxiety.

Unexpectedly, task order selectively impacted startle in the shock task. Overall startle magnitudes were larger during the shock task compared to during the screaming female task, but only when the shock task was completed first. In contrast, there were no differences in overall startle responses when the screaming female task was completed first. These results suggest that, when it was completed first, the shock UCS paradigm produced greater levels of baseline anxiety than the screaming female UCS. This finding is consistent with previous research demonstrating that a shock is a more potent UCS for context conditioning than other less aversive stimuli (e.g., airpuff to the larynx; Grillon et al., 2004). However, the potency of an electric shock may be mitigated when participants have already completed another fear learning task, potentially due to habituation.

In addition, FPS magnitudes in the two tasks were only correlated when the shock task was completed first. Related to the baseline (or overall) startle differences discussed above, it is possible that the shock task loses potency when completed second and consequently does not demonstrate the same association with other measures of fear learning. Taken together, these results seem to suggest that the shock UCS is more aversive and relates to other measures of fear learning, but only when completed first.

Importantly, there are several limitations of the current study that warrant discussion and suggest areas for future research. First, it is important to note the following differences between the two fear learning paradigms that could have impacted the results. (1) The electric shock is a vibrotactile UCS that activates peripheral nociceptors as well as mechanoreceptors, whereas the screaming female UCS does not. In a series of studies comparing auditory and tactile unconditioned stimuli, Cook et al. (1986) found that a combined tactile-noise UCS (i.e., loud noise combined with vibratory stimulus to the hand) produced superior fear conditioning compared to the auditory UCS alone. Although this combined vibration-noise UCS was not directly compared to a shock UCS, results from Cook et al. suggest that the tactile nature of the shock UCS may be central to its utility in fear learning. (2) The shock was a single UCS whereas the screaming female UCS includes both visual and auditory components. In the current study, it does not appear that this additional sensory modality increased the efficacy of the screaming female UCS. Nevertheless, future studies may consider matching the number of sensory modalities between UCS paradigms. Moreover, it will be helpful for future studies to examine whether the combined UCS, including both the fearful face and scream, are necessary for fear learning to occur. (3) In addition to different UCSs, the two tasks also employed different CSs: the shock task utilized geometric shape CSs and the scream task used female faces. Future studies may consider using a more sensitive design by including the same type of CSs when comparing fear learning paradigms. (4) The screaming female task included a more clearly matched, or associated, CS and UCS (i.e., two female faces and a related scream). Stronger CS–UCS associations have been shown to increase fear acquisition (see review: Ohman and Mineka, 2001). Again, although it did not increase the effectiveness of the UCS paradigm in the current study, this may be important for future research to consider. (5) Because participants chose their own level of shock, they received more shocks prior to initiation of the shock task than screams prior to the screaming female task. However, this additional exposure to the shock UCS likely resulted in habituation to the stimulus (Badia and Harley, 1970) rather than aided in fear acquisition using this UCS. (6) In the shock paradigm, the UCS is slightly different for each person (because each participant selects his/her own level

of shock), whereas the screaming female UCS is identical across participants. Therefore, future studies may consider selecting an alternative UCS that is also stable across participants.

In addition, the current study compared the screaming female UCS to one alternative UCS, an electric shock. Future research could consider comparing the efficacy of the screaming female paradigm to other UCSs, such as an air puff to the larynx, which is an alternative UCS commonly used with children (Grillon et al., 1999, 2005).

Finally, there are limitations related to the measurement of trait anxiety in the current sample. The trait anxiety measure was completed last and may have been affected by state-level increases in anxiety following the fear learning tasks. In addition, the current study included an unselected, nonclinical sample of college students, which provided a restricted range of anxiety symptoms. An association between trait anxiety and FPS using the screaming female UCS paradigm may have emerged within a larger, or more clinically severe, sample.

In conclusion, the present study is the first to directly compare the efficacy of the screaming female UCS paradigm to the standard shock UCS paradigm, using both FPS and self-report measures. The direct comparison of this relatively new UCS paradigm to the more standard shock UCS design is important given the increasing use of the screaming female UCS in fear learning studies with children and adolescents. Consistent with procedures used to validate other measures, such as self-reports, the current study examined the convergent validity of the scream UCS with an existing paradigm reliably used in fear learning research. Overall findings indicate that, although fear conditioning was observed using both UCSs, the shock UCS compared to the scream UCS was more: (1) aversive, (2) sensitive to individual differences in trait anxiety, and (3) related to self-reported fear learning. Key differences between these two UCSs (discussed above) may suggest ways to increase the potency of the scream UCS (e.g., including a vibrotactile component). And importantly, more studies are needed comparing the screaming female UCS to other UCSs typically used with children and adolescents.

## References

- Badia, P., Harley, J.P., 1970. Habituation and temporal conditioning as related to shock intensity and its judgment. *Journal of Experimental Psychology* 84, 534–536.
- Borelli, J.L., Sbarra, D.A., Crowley, M.J., Mayes, L.C., 2011. Mood symptoms and emotional responsiveness to threat in school-aged children. *Journal of Clinical Child and Adolescent Psychology* 40, 220–232.
- Britton, J.C., Lissek, S., Grillon, C., Nocrass, M.A., Pine, D.S., 2010. Development of anxiety: the role of threat appraisal and fear learning. *Depression and Anxiety* 0, 1–13.
- Brown, J.S., Kalish, H.L., Farber, I.E., 1951. Conditioned fear as revealed by the magnitude of startle response to an auditory stimulus. *Journal of Experimental Psychology* 41, 317–327.
- Cook, E.W., Hodes, R.L., Lang, P.J., 1986. Preparedness and phobia: effects of stimulus content on human visceral conditioning. *Journal of Abnormal Psychology* 95, 195–207.
- Craske, M.G., Waters, A.M., Bergman, R.L., Naliboff, B., Lipp, O.V., Negoro, H., Ornitz, E.M., 2008. Is aversive learning a marker of risk for anxiety disorder in children? *Behaviour Research and Therapy* 46, 954–967.
- Davis, M., Falls, W.A., Campeau, S., Kim, M., 1993. Fear-potentiated startle: a neural and pharmacological analysis. *Behavioural Brain Research* 58, 175–198.
- Glenn, C.R., Klein, D.N., Lissek, S., Britton, J.C., Pine, D.S., Hajcak, G., 2012. The development of fear learning and generalization in 8 to 13 year-olds. *Developmental Psychobiology* 54, 675–684.
- Grillon, C., 2002. Startle reactivity and anxiety disorders: aversive conditioning, context, and neurobiology. *Biological Psychiatry* 52, 958–975.
- Grillon, C., Davis, M., 1997. Fear-potentiated startle conditioning in humans: explicit and contextual cue conditioning following paired vs. unpaired training. *Psychophysiology* 34, 451–458.
- Grillon, C., Dierker, L., Merikangas, K.R., 1998. Fear-potentiated startle in adolescent offspring of parents with anxiety disorders. *Biological Psychiatry* 44, 990–997.
- Grillon, C., Merikangas, K.R., Dierker, L., Snidman, N., Arriaga, R.I., Kagan, J., Donzella, B., Dikel, T., Nelson, C., 1999. Startle potentiation by threat of aversive stimuli and darkness in adolescents: a multi-site study. *International Journal of Psychophysiology* 32, 63–73.
- Grillon, C., Baas, J.P., Lissek, S., Smith, K., Milstein, J., 2004. Anxious responses to predictable and unpredictable aversive events. *Behavioral Neuroscience* 118, 916–924.
- Grillon, C., Warner, V., Hille, J., Merikangas, K.R., Bruder, G.E., Tenke, C.E., Nomura, Y., Leite, P., Weissman, M.M., 2005. Families at high and low risk for depression: a three-generation startle study. *Biological Psychiatry* 57, 953–960.

- Grillon, C., Lissek, S., Rabin, S., McDowell, D., Dvir, S., Pine, D., 2008. Increased anxiety during anticipation of unpredictable but not predictable aversive stimuli as a psychophysiological marker of panic disorder. *American Journal of Psychiatry* 165, 898–904.
- Hamm, A.O., Greenwald, M.K., Bradley, M.M., Lang, P.J., 1993. Emotional learning, hedonic changes, and the startle probe. *Journal of Abnormal Psychology* 102, 453–465.
- Lang, P.J., Bradley, M.M., Cuthbert, B.N., 1990. Emotion, attention, and the startle reflex. *Psychological Review* 97, 377–395.
- Lau, J.Y.F., Lissek, S., Nelson, E.E., Lee, Y., Roberson-Nay, R., Poeth, K., et al., 2008. Fear conditioning in adolescents with anxiety disorders: results from a novel experimental paradigm. *Journal of the American Academy of Child and Psychiatry* 47, 94–102.
- Liberman, L.C., Lipp, O.V., Spence, S.H., March, S., 2006. Evidence for retarded extinction of aversive learning in anxious children. *Behaviour Research and Therapy* 44, 1491–1502.
- Lissek, S., Baas, J.M., Pine, D.S., Orme, K., Dvir, S., Nugent, M., Rosenberger, E., Rawson, E., Grillon, C., 2005a. Airpuff startle probes: an efficacious and less aversive alternative to white-noise. *Biological Psychology* 68, 283–297.
- Lissek, S., Powers, A.S., McClure, E.B., Phelps, E.A., Woldehawariat, G., Grillon, C., Pine, D.S., 2005b. Classical fear conditioning in the anxiety disorders: a meta-analysis. *Behaviour Research and Therapy* 43, 1391–1424.
- Mineka, S., Oehlberg, K., 2008. The relevance of recent developments in classical conditioning to understanding the etiology and maintenance of anxiety disorders. *Acta Psychologica* 127, 567–580.
- Neumann, D.L., Waters, A.M., Westbury, H.R., Henry, J., 2008. The use of an unpleasant sound conditional stimulus in an aversive conditioning procedure with 8- to 11-year-old children. *Biological Psychology* 79, 337–342.
- Ohman, A., 1986. Face the beast and fear the face: animal and social fears as prototypes for evolutionary analyses of emotion. *Psychophysiology* 23, 123–145.
- Ohman, A., Mineka, S., 2001. Fears, phobias, and preparedness: toward an evolved module of fear and fear learning. *Psychological Review* 108, 483–522.
- Reeb-Sutherland, B.C., Helfinstein, S.M., Degnan, K.A., Perez-Edgar, K., Henderson, H.A., Lissek, S., et al., 2009. Startle response in behaviorally inhibited adolescents with a lifetime occurrence of anxiety disorders. *Journal of the American Academy of Child and Psychiatry* 48, 610–617.
- Schmitz, A., Merikangas, K., Swendsen, H., Cui, L., Heaton, L., Grillon, C., 2011. Measuring anxious responses to predictable and unpredictable threat in children and adolescents. *Journal of Experimental Child Psychology* 110, 159–170.
- Seligman, M.E.P., 1971. Phobias and preparedness. *Behavior Therapy* 2, 307–320.
- Sesti, A., 2000. State trait anxiety inventory (STAI) in medication clinical trials. *Quality of Life Newsletter* 25, 16–17.
- Spielberger, C.D., Gorsuch, R.L., Lushene, R.E., 1970. *Trait Anxiety Inventory (Self Evaluation Questionnaire)*. Consulting Psychologist Press, Palo Alto, CA.
- Tottenham, N., Tanaka, J.W., Leon, A.C., McCarry, T., Nurse, M., Hare, T.A., et al., 2009. The NimStim set of facial expressions: judgments from untrained research participants. *Psychiatry Research* 168, 242–249.
- Waters, A.M., Henry, J., Neumann, D.L., 2009. Aversive Pavlovian conditioning in childhood anxiety disorders: impaired response inhibition and resistance to extinction. *Journal of Abnormal Psychology* 118, 311–321.