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Human fear conditioning and extinction: Timing is everything . . . or is it?

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Abstract

A differential fear conditioning paradigm was used with 107 healthy undergraduate participants to evaluate the effect of conditioned stimulus (CS) temporal properties on fear acquisition and extinction. Two minute duration CSs were used for Day 1 fear acquisition. Participants were randomized to receive either 1, 2, or 4 minute CS durations during Day 2 extinction. Extinction re-test was examined on Day 3 using the original acquisition CS duration (2 minutes). Findings indicated that participants who were aware of the CS+/unconditioned stimulus (US) contingency ($n=52$) develop a temporal expectation about when the unconditioned stimulus will be delivered. Although the shorter duration CS resulted in greater fear reduction during extinction, cessation of fear responding at re-test was the same for CS extinction durations ranging from half the CS acquisition duration to twice the CS acquisition duration. Thus, extinction performance did not predict extinction at re-test, which could have important implications for optimizing exposure therapy for anxiety disorders.

Keywords

fear conditioning; anxiety; psychophysiology; temporal conditioning; stimulus duration

Although anxiety disorders are effectively treated by exposure therapy (e.g., Norton & Price, 2007) there is room for improvement as not all individuals are appreciably helped by treatment (e.g., Blanchard et al., 2004) and some of those who are helped suffer a relapse of anxiety at a later date (e.g., Rachman, 1989). Because exposure therapy is believed to operate in part through extinction processes (Bouton, Mineka, & Barlow, 2001; Mineka & Zinbarg, 2006), better understanding of fear extinction mechanisms will help to optimize exposure therapy parameters which in turn could lead to more efficacious treatments. Specifically, those with anxiety disorders may develop a temporal expectation about the

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aversive event they expect in response to their feared stimulus. Understanding the role this temporal expectation plays in extinction of conditioned fear could help therapists to optimize exposure durations. Thus, the present study examined the effect of different duration conditional stimuli (CSs) during extinction on reduction of conditioned fear in humans.

In the present study, extinction will refer to the process underlying fear reduction as well as the experimental phase associated with this process. Reduction of fear observed within the extinction phase will be referred to as extinction *performance*, whereas fear reduction observed at some duration after extinction will be referred to as extinction *re-test*. Laboratory extinction performance is akin to short-term fear reduction seen during therapy exposures. In addition to short-term relief, exposure therapy also aims to optimize long-term outcomes, such as relapse prevention, which are more akin to extinction re-test. It is important to differentiate between the two because extinction performance does not guarantee extinction at re-test and the absence of extinction performance does not preclude extinction at re-test (Plendl & Wotjak, 2010).

The mechanisms by which extinction occurs are still under investigation. Although early theorists believed that extinction resulted in ‘unlearning’ of the originally learned relationships (e.g., Rescorla & Wagner, 1972), there is evidence to indicate that original learning still exists after extinction (see Bouton, 2002). Thus, one current associative account proposes that extinction is new, inhibitory learning (CS+/no US) that competes with the original, excitatory CS+/US learning (e.g., Bouton, 1993); an alternative explanation involves an inhibitory association between the CS+ and the particular conditioned response (e.g., Rescorla, 1997).

Although of debatable *necessity* (for a review, see Lovibond & Shanks, 2002), expectancy of the US in response to the CS+ is central to human fear conditioning as conditioned responding only occurs for those aware of the CS+/US contingency (e.g., Prenoveau, Craske, Liao, & Ornitz, in press; Purkis & Lipp, 2001). As excitatory learning is based in part on the expectancy of the US in response to the CS+, inhibitory learning, or extinction, is posited to occur from a violation of this expectancy. Additionally, for fear acquisition, there is evidence that expectancy of the US in response to the CS+ is temporally specific. For example, in fixed delay fear conditioning with animals, maximal CRs generally occur at, or around, the time of US delivery (e.g., Drew, Zupan, Cooke, Couvillon, & Balsam, 2005). Thus, timing likely plays a role in extinction and the violation of US expectancy in response to the CS+. Theories of conditioning differ as to what that role might be.

It is possible that extinction performance and extinction re-test are temporally cue specific. In other words, the less similar the extinction CS+ is to the acquisition CS+, the less generalization of fear there will be from the acquisition to extinction CS+. Thus, the more dissimilar the extinction CS+ duration is to the acquisition CS+ duration (either longer or shorter in duration), the easier it will be to extinguish fear to the CS+ during extinction. Therefore, an enhancement of extinction performance will be observed as the extinction CS+ duration becomes less similar to the acquisition CS+ duration. Under a temporal cue-specificity model although extinction *performance* would be improved as a result of using a more dissimilar CS+ duration during extinction, extinction *re-test* would suffer. The use of a different duration CS+ during extinction would result in a generalization gradient at extinction re-test: the less similar the extinction CS+ duration was to the acquisition CS+ duration, the less effective extinction will be at reducing responding at re-test (extinction re-test generalization decrement: attenuated reduction in responding due to generalization). Such generalization gradients for extinction performance and re-test have been shown in appetitive paradigms using auditory tones of different frequencies (as opposed to different

durations) (e.g., Dubin & Levis, 1973). A similar pattern has also been observed in human fear conditioning using geometric shapes as CSs (Vervliet, Vansteenwegen, & Eelen, 2004).

Componential trace models that view the CS+ as multiple successive cues that can acquire differential associations with the US (see Brandon, Vogel, & Wagner, 2002). Such models posit that the earlier CS cues acquire inhibitory properties whereas later cues which are present for US delivery become excitatory; thus, the earlier CS+ cues would need to be present during extinction in order for the later excitatory cues to be encountered. Such models would predict that the CS+ must be displayed as long, or longer, than that of the acquisition CS+ in order to optimize extinction re-test. An extinction re-test decrement would not be seen for a longer duration CS+, but would be observed for an extinction duration shorter than that of acquisition.

Rate expectancy theory (RET, Gallistel & Gibbon, 2000) posits that extinction is based on a ratio of cumulative time exposed to the CS+ during extinction to the anticipated amount of CS+ exposure per US reinforcement. Thus, the number of extinction trials by itself is unimportant, as is the duration of each individual trial; extinction learning is solely based on prior acquisition learning and cumulative CS+ exposure during extinction. In contrast, other models posit that expectation of US delivery is timed from the CS+ *onset*, regardless of whether or not the CS+ continues to be displayed (e.g., Grossberg & Schmajuk, 1991). Such models predict that extinction is solely based on the number of extinction CS+ presentations, regardless of duration.

Findings from animal fear conditioning studies have been mixed with some supporting RET for extinction performance (Plendl & Wotjak, 2010) and extinction re-test (Shipley, 1974), and others supporting temporal cue specificity and a generalization decrement at extinction retest (Plendl & Wotjak, 2010). Findings from animal appetitive paradigms have also been mixed. Some findings have been consistent with cue specificity and generalization decrement for extinction performance (Haselgrove & Pearce, 2003) and extinction re-test (Drew, Yang, Ohyama, & Balsam, 2004), whereas others have been consistent with componential trace and CS+ onset timing models for extinction performance (Drew et al., 2004). Although such models have not been tested in human fear conditioning paradigms, there is evidence consistent with componential trace models that for phobic samples a few (or a single) long exposure(s) are more effective than multiple short exposures (e.g., Chaplin & Levine, 1981; Marshall, 1985). However, such studies have not specifically manipulated exposure lengths in an attempt to violate temporal expectancies of an aversive outcome.

A better understanding of which model is most applicable to human fear extinction has clear implications for the treatment of anxiety disorders. If extinction is temporally cue specific, therapists would want the temporal properties of exposure to closely match those of the client's learning in order to optimize long-term outcomes. Under this model, exposures that are temporally shorter or longer than a client's learning might appear more efficacious during therapy (enhanced extinction performance), but result in poorer long-term outcomes (extinction re-test generalization decrement). If CS+ onset models were found to predominate, then therapists would be much more concerned about the number of exposures as compared to their duration. Thus, the exposure duration for optimizing long-term therapeutic outcomes would depend on which model is found to best represent extinction processes.

Given the theoretical importance of understanding the effect of CS+ temporal properties on extinction and the clinical impact such understanding could have on exposure therapy, it is surprising how little research has been conducted to elucidate these parameters. Thus, the present study used a human differential fear conditioning paradigm to evaluate temporal CS

+US expectancies and their impact on extinction. Specifically, 2 min duration CSs were used during Day 1 fear acquisition to evaluate if expectancy of the US in response to the CS + is temporally specific in humans as in animals (e.g., Drew et al., 2005). To evaluate the effects of varying extinction CS duration on extinction performance, participants were randomized to receive either 1, 2, or 4 min CS durations during Day 2 extinction. Two min CS durations were again used for Day 3 follow-up to evaluate the impact of the manipulation on extinction re-test. Magnitude of associative fear conditioning and subsequent extinction performance and re-test was assessed through both self-report (fear of CSs and online US expectancy during CSs) and physiological indices (skin conductance response and startle reflex).

Method

Participants

One-hundred and seven undergraduates at the University of California at Los Angeles (UCLA) participated for course research credit or payment of sixty dollars. Data from 27 participants was excluded because they discontinued during Day 1 ($n = 3$), did not return for or complete Day 2 ($n = 19$), or there were technical problems ($n = 5$). An additional 28 were excluded from analyses as they were unaware of the CS+/US contingency (see **Contingency Awareness** below for details). The 52 remaining participants ranged in age from 18.3 to 22.8 ($M = 19.7$, $SD = 1.1$) and consisted of 25 females (48.1%). Self-endorsed ethnic breakdown was as follows: 36.5% Asian, 38.5% Caucasian, 15.4% Hispanic/Latin American, and 9.6% reported a mix of ethnicities. All participants were provided with a description of the study and gave written, informed consent that had been approved by UCLA's institutional review board.

Materials

Self-Report Measures—An 11-point Likert scale was used to obtain subjective fear ratings of the CS+ and CS− (0 = ‘not at all fearful of’, 10 = ‘very fearful of’). Participants’ expectancy of receiving the US was rated during CS presentations and intertrial intervals (ITIs) by using a joystick to move an on-screen pointer along an analog scale between the extremes of 0 = ‘certain no stimulation’ and 10 = ‘certain stimulation’ with a midpoint of 5 = ‘uncertain’. The scale appeared onscreen at specific times (see **Procedure** for details) prompting participants to make a rating based on their expectancy of receiving the US in “the next few moments”. A short recognition questionnaire (Dawson & Reardon, 1973) was administered after acquisition to assess awareness of the CS+/US contingency: participants were asked what the muscle stimulation was paired with: the green triangle, the purple trapezoid, the movie, there was no pattern, they could not tell, or there were no muscle stimulations. They were then asked how sure they were: completely uncertain, fairly uncertain, fairly certain, or completely certain.

Physiological Measures and Startle Probes—Skin conductance responses (SCRs) to CS onsets served as an index of CS association with the US. Eye blink startle reflex (SR) during CS presentations and ITIs served as an indicator of defensive emotional response; the SR can be considered an index of fear to a specific cue. The startle probes, acoustic startle stimuli delivered to elicit SRs, consisted of 50-ms, 104 dB bursts of “white noise” with an instantaneous rise time delivered binaurally via stereophonic headphones. SRs were measured by electromyogram (EMG) activity of the orbicularis oculi (recorded from two 4 mm Ag/AgCl electrodes). Eye blink SR magnitude was calculated as the difference between the mean EMG level during 200 ms immediately preceding the startle stimulus and the peak response, in microvolts (μV). SRs for a given startle stimulus were rejected when tonic EMG background exceeded a preset criterion or vertical EOG revealed excessive gaze

shifts, spontaneous blinks, or saccades during the 200 ms pre-startle period. SRs were also rejected if blink response onset was earlier than 20 ms following startle stimulus onset or if behavioral observations indicated gross body movement or drowsiness prior to startle stimulus delivery. SRs were scored as zero if no onset latency was identified within the 20–80 ms window. Natural log transforms were used for SR analyses to correct for statistical non-normality of data.

SCRs were recorded from two 3 mm diameter Ag/AgCl electrodes placed on the distal phalanx of the index and middle fingers of the non-dominant hand. The magnitude of SCRs were calculated as the difference between the trough and apex of the skin conductance level curve, expressed in microsiemens (μ S), commencing within 1–4 s following CS onset. SCRs were rejected for a given CS presentation if behavioral observations or other physiological measures indicated excessive drowsiness, movement, or behavior such as coughing and sneezing. SCRs were scored as zero for a given CS presentation when there was no observable SCR activity commencing within the 1–4 s window. Physiological data were acquired using a Grass Instruments Amplifier System and were digitized and sampled at 1000 Hz. EMG was full-wave rectified and AC amplified at a gain of 10,000. Low and high frequency cut-off values were 30 Hz and 1000 Hz for EMG. SCR was DC amplified at a gain of 2,000.

Apparatus, CSs, and US—Conditional stimuli, ITI movie display, muscle stimulation delivery, expectancy ratings, and recording of physiological activity were under the control of National Instruments LabVIEW Programming Software (v7). CSs consisted of a green triangle or purple trapezoid displayed on a 21 inch computer monitor located 3 feet from participants at eye level (see **Procedure** for details about number and duration of CSs). Bicep muscle stimulations which served as the aversive US consisted of 20.4 mA peak current (equating to a 50 V peak) passing between two pads for 0.5 s and was delivered by a Digital 807 Electrical Muscle Stimulation Device (Everyway Medical Instruments). Such stimulation results in a rapid onset, involuntary muscle contraction across the biceps. The intensity level of the stimulation was preset based on pilot testing to a level that was considered uncomfortable but not painful.

Procedure—Participants were asked to participate in three sessions conducted on three consecutive days with start times differing by no more than four hours. Differential fear conditioning was conducted on Day 1. An extinction phase where participants were randomly assigned to one of three groups (1-, 2-, or 4-min CS durations) was conducted on Day 2 and extinction re-test was assessed on Day 3. See Figure 1 for an overview of the experimental procedure including number and duration of CSs and ITIs for the various phases and groups (additional details also provided below).

On Day 1, a trained research assistant attached participant electrodes, verified impedances were below 20 Kohms, and administered a hearing test to ensure participants could adequately hear startle tones. Participants were taught how to rate their expectancy of receiving a muscle stimulation and practiced doing so. Muscle stimulation pads were attached to participants' bicep and it was explained that they would be receiving stimulations through these pads. Participants were instructed they would *not* be receiving muscle stimulations during the next 20 mins (baseline). Baseline assessed expectancy and physiological reactivity to the 'to be' CSs and ITIs between CSs. A movie without sound was displayed during ITIs for all phases. A movie was shown during ITIs in order to maintain participant attention during 10 min Day 2 ITIs; the movie was also shown during Days 1 and 3 to prevent a contextual change. Baseline consisted of 2 'to be' CS+ trials and 2 'to be' CS− trials, displayed in random order. A 'trial' refers to a single presentation of the CS onscreen (from onset to offset) regardless of duration. The 'to be' CS+ and CS− were 2-

min duration images of a green triangle or purple trapezoid (counterbalanced across participants). During each CS, two online expectancy ratings (early and late) were prompted through presentation of the analog scale at 48 and 108 s post CS onset. An early and late startle probe was delivered during each CS, with delivery randomized during the periods of 34 to 38 s ($M = 36$ s) and 94 to 98 s ($M = 96$ s) post CS onset. ITIs were 90 s in duration with the expectancy scale appearing 48 s post CS offset and a startle probe being delivered between 34 and 38 s ($M = 36$ s) post CS offset (expectancy rating and startle probe timing is illustrated in Figure 2).

Pre-acquisition state anxiety was assessed after baseline. Participants were instructed they *may* experience muscle stimulations during the next experimental phase; they were not informed of a CS-US relationship. The acquisition phase differed from baseline in two ways: first, during CS+ presentations a mildly unpleasant biceps muscle stimulation (the US) was delivered 117 s post CS+ onset (3 s prior to offset). Second, rather than 2 trials of each CS, there were 4 CS+ and 4 CS– trials. After all 8 acquisition trials, participants provided CS subjective fear ratings before completing the recognition questionnaire.

On Day 2, subjects underwent extinction. After electrodes and muscle stimulation pads were attached, participants were instructed they *may* experience muscle stimulations during the next experimental phase; no muscle stimulations were delivered on Day 2. Participants were randomized to one of three groups, which differed on the length of CS presentations as well as number of CS trials (to equate total CS exposure). Participants provided pre-extinction CS subjective fear ratings after electrodes and muscle stimulation pads were attached and before the movie or CSs were displayed. As detailed in Figure 1, the number and duration of CS trials for the three groups was: 1-min group: 8, 1-min CSs (4 CS+, 4 CS–); 2-min group: 4, 2-min CSs (2 CS+, 2 CS–); 4-min group: 2, 4-min CSs (1 CS+, 1 CS–). As shown in Figure 2, the number of expectancy ratings and probes per time of CS display was held constant across groups. Thus, the total number of startle probes (4) and expectancy ratings (4) during Day 2 CSs were equal across groups. ITI duration for all three groups was 10 min with three startle probes and three expectancy ratings during each ITI.

Because the number of ITIs varied with group, participants were randomly assigned to either a pre- or post-extinction period (PEP) of 40 mins (2-min group) or 60 mins (4-min group) to equalize time spent in the context. PEPs were organized into 10 min blocks that were identical to Day 2 ITIs, with 3 startle probes and 3 expectancy ratings given at the same times as during ITIs. Thus, on Day 2 participants in all 3 groups were in the context for the same duration (78 min), viewed the movie for the same duration (70 min), and received the same number of startle probes (21) and expectancy ratings (21) during movie presentation. At the completion of extinction (or the post-extinction period), participants provided post-extinction CS subjective fear ratings.

Extinction re-test was conducted on Day 3. After electrodes and muscle stimulation pads were attached, participants provided re-test CS subjective fear ratings. Prior to re-test, participants were instructed they *may* experience muscle stimulations during the next experimental phase. Re-test CS trials were the same duration as acquisition (2-min) and there were 2 trials of each CS. The US was not delivered during the CS+ on Day 3. The timing and number of startle probes and expectancy prompts for CSs and ITIs were identical to that of acquisition (see Figure 2).

Results

Contingency Awareness

Participants who indicated on the post-acquisition questionnaire they were ‘completely’ or ‘fairly’ certain that the US was paired with the CS+ were considered contingency aware. Although this method is consistent with past research (e.g., Grillon, 2002), it does not account for the fact that participants who did not accurately select the CS+ on the questionnaire may have failed to do so for reasons other than an unawareness of the CS+/US contingency, such as lack of confidence about their perceptual experience or retrospective biases in reporting (e.g., Lovibond & Shanks, 2002). Thus, online expectancy ratings to CSs were used to identify participants who answered the post-acquisition questionnaire incorrectly, but who were likely aware of the CS+/US contingency. Participants’ differential expectancy, or CS+ expectancy minus CS– expectancy, was calculated for pre-acquisition and acquisition. If participants’ acquisition differential expectancy exceeded zero by two standard deviations (based on the pre-acquisition distribution), it was deemed highly unlikely that they were unaware of the contingency.

Of the 80 participants with no technical problems who completed all three days, 52 (65%) were designated aware of the CS+/US contingency based on the criteria above. Although the percentage of aware participants did not differ by Group (1-min, 2-min, and 4-min extinction CS durations), $\chi^2(2) = 5.34$, $p = 0.07$, unaware participants were excluded from analyses because the present study sought to determine the effect of extinction on those who learned the CS+/US association. Further, the pattern of results described below for those who were considered contingency aware ($n = 52$) was virtually identical to that observed for all participants who completed the 3 days without technical problems ($n = 80$). Generally, effect sizes for the contingency aware group (presented here) were larger than those for all participants who completed the 3 days without technical problems.

Fear Conditioning

It was not expected that there would be between group differences for Day 1 fear conditioning as individuals in different Groups were not treated differently until Day 2. Consistent with this, no significant main or interactive effects involving Group (all $F < 2.7$, $p > 0.09$) were found for any dependent variables during acquisition when Group $\times$ CS-type (CS– and CS+) $\times$ Time (early CS and late CS) $\times$ Trial (acquisition trials 1–4) repeated measures analyses of variance (ANOVAs) were conducted with Group as a between-subjects factor. Thus, acquisition data (dependent variable means and standard errors) are presented for all participants, collapsed across Group in Figure 3.

For dependent variables where Time could not be examined as a factor (subjective fear ratings and SCRs), it was hypothesized that participants would learn to fear the CS+ to a greater extent than the CS– during acquisition. As seen in Figure 3b, this was seen for subjective fear ratings: the difference between CS+ and CS– prior to acquisition was not significant, $F(1,49) = .3$, $p = .64$, but subjective fear to the CS+ was significantly greater than that to the CS– post-acquisition, $F(1,49) = 63.0$, $p < .001$, $\eta^2 = .56$; the expected CS-type $\times$ Phase (pre-acquisition and post-acquisition) interaction was significant, $F(1,49) = 56.6$, $p < .001$, $\eta^2 = .54$. As seen in Figure 3d, this same pattern was observed for SCRs: the difference between CS+ and CS– for Trial 1 was not significant $F(1,24) = .01$, $p = .92$, but SCRs to the CS+ were significantly greater than that to the CS– by the last trial, $F(1,24) = 11.1$, $p = .003$, $\eta^2 = .32$. However, the expected CS-type $\times$ Trial interaction did not reach significance for SCRs, $F(3,72) = 1.2$, $p = .32$.

For dependent variables where Time could be examined as a factor (US expectancy ratings and SRs) it was hypothesized that participants would develop a temporal expectation of the

US during acquisition for the CS+, but not the CS-. In other words, fear would not increase during the course of the CS at the beginning of acquisition for either CS+ or CS-, but fear would increase from early to late in the CS trial for only the CS+ by the end of acquisition. As seen in Figure 3a, this pattern was observed for US expectancy ratings; the expected CS-type $\times$ Time $\times$ Trial interaction was significant, $F(3,114) = 21.3$, $p < .001$, $\eta^2 = .36$. Within CS+ trials, there was no change in US expectancy from the early to late time period for the first CS+ acquisition trial, $F(1,38) = .35$, $p = .56$, but there was a large, significant increase for the final CS+ trial, $F(1,38) = 73.0$, $p < .001$, $\eta^2 = .66$. The expected CS-type $\times$ Time $\times$ Trial finteraction did not reach the level of significance for SRs, $F(3,69) = .5$, $p = .69$. However, as seen in Figure 3d, SRs generally decreased during the course of CSs, but for the CS+, this decrease turned into a slight increase by the end of acquisition. In fact, examination of simple effects for the final acquisition trial revealed there was no significant difference in SRs between the CS+ and CS- at the early time point, $F(1,40) = 0.1$, $p = .82$, but the difference approached significance at the late timepoint, $F(1,40) = 3.3$, $p = .07$, $\eta^2 = .08$.

Extinction Performance

Because Groups have different number of trials and trial durations during extinction, comparisons for expectancy ratings and SRs are based on duration of CS exposure rather than number of trials. The majority of models discussed above would indicate that there would be a significant difference in change in fear responding to the CS+ across extinction among the Groups; the only exception would be RET because the present study holds total duration of exposure constant across Groups. Thus, it was hypothesized that the Groups would show a significant difference in the amount of fear reduction to the CS+ during extinction.

As seen in Figure 4a, this was the case for US expectancy ratings to the CS+: the 1-min group showed a significant decrease during extinction, whereas the 2- and 4-min group did not show a significant change; the Group $\times$ Duration (48 s, 108 s, 168 s, and 228 s) interaction was significant, $F(6,132) = 4.3$, $p = .001$, $\eta^2 = .16$. Also, whereas the 2- and 4-min groups continued to display differential conditioning after 168 s of exposure to both CS+ and CS-, both $F(1,46) > 10.6$, $p < .002$, $\eta^2 > .19$, the 1-min group did not, $F(1,46) = 3.1$, $p = .08$. Data for the CS- is not provided in Figure 4 for clarity of presentation, but is available upon request from the first author. As can be seen in Figures 3b, 3c, and 3d, the reduction in fear responding to the CS+ during extinction appeared to be largest for the 1-min group as compared to the 2- and 4-min groups. However, the Group $\times$ CS-Type $\times$ Phase interaction did not reach the level of significance for subjective fear, SRs, or SCRs (all $F < 2.7$, $p < .12$). It is possible that significant differences among the groups exist, but the present sample size was underpowered to detect them. In fact, when examining simple effects, almost the same pattern of results was seen for SCRs and SRs as US expectancy. The 1-min group showed a significant reduction in responding during extinction for SRs, $F(1,40) = 5.1$, $p = .03$, $\eta^2 = .11$, and nearly showed one for SCRs, $F(1,20) = 3.7$, $p = .07$, $\eta^2 = 0.15$, whereas neither the 2- nor 4-min groups showed significant changes in SRs or SCRs during extinction (all $F < 3$, $p > .10$).

Next, US expectancy and SRs were evaluated to determine if the temporal expectation of the US acquired during Day 1 persisted to Day 2, and whether or not US delivery was timed from CS+ onset regardless of whether or not the CS+ continued to be displayed. If the temporal expectation of US delivery persisted from Day 1 to Day 2, then the temporal pattern of fear responding to the CS+ should not change for the 2-min group from the end of acquisition to the start of extinction. In support of retention of temporal expectation of US delivery from Day 1 to Day 2, the 2-min group did not display a significant Trial (acquisition trial 4 and extinction trial 1) $\times$ Time (48, 108, and 168 s post CS+ onset)

interaction for either CS+ US expectancy or SRs, both $F < 1.0$, $p > .05$. As seen when comparing Figures 5a and 5b for the 2-min group, there was no change in temporal expectation of the US from Day 1 to Day 2: US expectancy increased significantly from 48 to 108 s post CS+ onset and then decreased significantly from 108 to 168 s post CS+ onset. In other words, as the CS+ was onscreen and the time of US delivery (117 s) neared, participants' expectancy of receiving the US increased, whereas it decreased after the CS+ was removed from the screen.

If expectancy of US delivery is timed from CS+ onset (regardless of whether or not the CS+ is displayed onscreen), both the 1- and 4-min Groups should also show the same temporal pattern of responding in extinction as they did in acquisition. However, this was not the case as can be seen when comparing Figures 5a and 5b for both the 1- and 4-min groups. Both the 1- and 4-min groups display significant Trial (acquisition trial 4 and extinction trial 1) $\times$ Time interactions, both $F > 15.8$, $p < .001$, $\eta^2 > .64$. For the 1-min group, US expectancy *increases* significantly from 48 to 108 s post CS+ onset when the CS+ was onscreen during acquisition (Figure 5a), $F(1,10) = 14.7$, $p = .003$, $\eta^2 = .59$, but *decreased* significantly from 48 to 108 s post CS+ onset when the CS+ was removed during this period for extinction (Figure 5b), $F(1,10) = 5.4$, $p = .04$, $\eta^2 = .35$. The pattern of SR means was similar to that of US expectancy ratings for the 1-min group, but the Trial $\times$ Time interaction was not significant, $F(1,9) = 1.1$, $p = .33$. For the 4-min group, there was a significant decrease from 108 to 168 s post CS+ when the CS+ was removed from the screen during this period for acquisition (Figure 5a), $F(1,9) = 27.7$, $p < .001$, $\eta^2 = .75$, but there was no significant change when the CS+ remained onscreen for this entire period during extinction, $F(1,9) = 0.4$, $p = .56$ (Figure 5b). For the 4-min group, the Trial $\times$ Time interaction for CS+ SRs was also significant, $F(2,12) = 6.8$, $p = .01$, $\eta^2 = .53$. As with US expectancy, there was a significant decrease in SRs from 108 to 168 s post CS+ onset for acquisition, $F(1,6) = 14.3$, $p = .009$, $\eta^2 = .71$, but not for extinction, $F(1,6) = 2.2$, $p = .19$.

Extinction Re-test

Based on the temporal cue specificity model, it was hypothesized that the reduction in differential fear conditioning from acquisition to extinction re-test would be larger for the 2-min group than either the 1- or 4-min groups. There was no support for this hypothesis as Group $\times$ CS-Type $\times$ Time $\times$ Trial (acquisition trial 4 and re-test trial 1) ANOVAs revealed that there were no significant Group main or interactive effects for US expectancy, SRs, or SCRs, all $F < 1.9$, $p > .16$ (see Figure 6a, 6c, and 6d)¹. The only significant effect of Group was seen for subjective fear ratings. A Group $\times$ CS-type $\times$ Phase (post-acquisition, extinction re-test) repeated-measures ANOVA for subjective fear ratings revealed a significant Group $\times$ Phase interaction, $F(2,48) = 3.3$, $p = .04$, $\eta^2 = .12$. Although subjective fear reduced significantly to both the CS+ and CS- for all three groups, the reduction for the 4-min group was slightly (yet significantly) larger than that of the 1- or 2-min groups when collapsing across CS-type. Given that the 4-min group had larger post-acquisition fear ratings, it is possible that this difference in combination with floor effects at re-test contributed to the observed interaction.

Next, US expectancy and SRs were evaluated to determine if the temporal expectation of the US acquired during acquisition persisted to extinction re-test and whether or not Day 2 extinction resulted in a decrease in fear responding at Day 3 re-test. A Group $\times$ CS-type $\times$

¹This lack of significant Group main or interactive effects was also found when conducting Group $\times$ CS-type $\times$ Time $\times$ Trial analyses for the following levels of Trial: Trial (acquisition trial 4, re-test trials 1 and 2), Trial (acquisition trial 4, re-test trial 2), and Trial (acquisition trial 4, average of re-test trials 1 and 2). Additionally, Group did not display any main or interactive effects when removing the CS-type factor and looking only at Group $\times$ Time $\times$ Trial analyses for the CS+ (for all Trial combinations discussed in the preceding sentence).

Time $\times$ Trial (acquisition trial 4 and re-test trial 1) ANOVA for US expectancy revealed a significant CS-type $\times$ Time $\times$ Trial interaction, $F(1,44) = 28.7$, $p < .001$, $\eta^2 = .40$. This interaction can be seen in Figure 6a: the temporal expectation of the US during the CS+ is demonstrated by US expectancy significantly increasing from the early to late time point at both the end of acquisition, $F(1,44) = 62.6$, $p < .001$, $\eta^2 = .59$, and the beginning of re-test, $F(1,44) = 15.7$, $p < .001$, $\eta^2 = .26$. However, the magnitude of this increase is significantly smaller at follow-up, $F(1,44) = 33.8$, $p < .001$, $\eta^2 = .43$, indicating a reduction in CS+ responding at re-test. The three-way interaction arises because unlike the CS+, there is no significant Time $\times$ Trial interaction for the CS- (see Figure 6a), $F(1,44) = 0.8$, $p = .37$. A Group $\times$ CS-type $\times$ Time $\times$ Trial ANOVA for SRs also revealed a CS-type $\times$ Time $\times$ Trial interaction, $F(1,32) = 5.7$, $p = .02$, $\eta^2 = .15$. As with US expectancy, this involved a significant Time $\times$ Trial interaction for the CS+, $F(1,32) = 5.1$, $p = .03$, $\eta^2 = .14$, but not for the CS-, $F(1,32) = 0.4$, $p = .51$ (see Figure 6c).

Subjective fear and SCRs were examined to determine if extinction resulted in a decrease in differential fear responding from acquisition to extinction re-test. It was hypothesized that such a reduction would be observed. As seen in Figure 6b, this was the case for subjective fear ratings; there was a significant Phase $\times$ CS-type interaction, $F(1,48) = 39.7$, $p < .001$, $\eta^2 = .45$. While subjective fear ratings decreased significantly from post-acquisition to extinction re-test for both the CS+, $F(1,48) = 73.0$, $p < .001$, $\eta^2 = .60$, and the CS-, $F(1,48) = 32.2$, $p < .001$, $\eta^2 = .40$, this decrease was significantly greater for the CS+. A similar pattern was seen for SCRs: there was a significant difference between CS+ and CS- SCRs at the end of acquisition, $F(1,33) = 9.0$, $p = .005$, $\eta^2 = .21$, but not at re-test trial 1, $F(1,33) = .3$, $p = .62$ (see Figure 6d). However, the CS-type $\times$ Trial interaction did not reach significance, $F(1,33) = 1.1$, $p = .30$.

Discussion

Using a human differential fear conditioning paradigm, the present study found several important effects relating to temporal US expectancy and the impact of varying CS+ extinction duration on the violation of this expectancy as reflected in extinction performance and re-test. Findings showed that in addition to developing an expectation of the US in response to the CS+, humans also develop a temporal expectation about when during the CS+ the US will be delivered². This temporal expectancy was specific to the CS+. Such findings are consistent with those from animal fear (e.g., Drew et al., 2005), animal appetitive (e.g., Ohyama, Gibbon, Deich, & Balsam, 1999) and human avoidance paradigms (e.g., Molet, Callejas-Aguilera, & Rosas, 2007). To our knowledge, this is the first time that such temporal specificity has been demonstrated in a human fear conditioning paradigm.

Also, for fixed total CS+ exposure, four 1-min extinction trials resulted in greater extinction performance than two 2-min trials or one 4-min trial. Thus, extinction performance was more a function of number of CS+ exposures than of cumulative exposure. Such findings are consistent with past work showing that extinction durations equal to or longer than that of acquisition performed similarly when CS+ total exposure was equated (Plendl & Wotjak, 2010). The present findings are also consistent with research where CS+ extinction durations

²As noted by a reviewer, an alternate explanation for the observed pattern of results is that it reflects the natural form of the conditioned response as opposed to reflecting participant knowledge about US timing. However, many human fear conditioning studies using short duration CSs (e.g., 8 s) demonstrate decreases in fear responding during the ITI and CS- compared to the CS+ (e.g., Grillon, 2002). For these studies, the ITI or CS- was as likely to be present as the CS+ at 108 s after the onset of a given CS+. Thus, the decreases in fear responding seen during the ITI and CS- for these studies would not have been observed if the natural form of a conditioned fear response takes the temporal pattern shown in the present study (increased fear at 108 s post CS+ onset). Thus, it seems likely that the increases in fear from early to late in the CS+ observed in the present study were the result of participants learning the timing of the US as opposed to being the result of the natural form of the conditioned response.

half that of acquisition resulted in faster within-session cessation of responding than CS+ extinction durations equal to or twice as long as that of acquisition (Drew et al., 2004). Drew and colleagues hypothesized that the more rapid reduction in extinction performance for the shorter CS+ was the result of generalization decrement: the extent of temporal dissimilarity between the acquisition and extinction CS+ impacts the rate of extinction performance. If this were the mechanism that resulted in faster extinction performance for the shorter duration, faster cessation of responding should also have been seen for the longer duration. However, this was not the case for the present study or for that of Drew and colleagues (for an exception, see Haselgrove & Pearce, 2003).

It could be argued that generalization impacts extinction performance for the shorter, but not longer duration CS+, because it is easier to differentiate between shorter CSs and a target CS than longer CSs and a target CS (e.g., Wearden, Denovan, & Haworth, 1997). However, this asymmetry is not seen when the CSs are temporally spaced in equal logarithmic intervals around the target CS (e.g., Wearden et al., 1997) as done in the present study and in Drew et al. (2004). It could be argued that generalization did not impact extinction performance for the 4-min group because it only consisted of one CS+ extinction trial and it may take multiple trials to learn the duration has changed. However, this argument does not hold for Drew and colleagues' findings because they delivered 64 trials during extinction.

Four 1-min CS+ trials resulting in greater extinction performance than two 2-min or one 4-min trial is consistent with CS+ onset timing models. Under this model, the 1-min group would have their expectancy of receiving the US at a given time after CS+ onset violated 4 times as compared to 2 times and 1 time respectively for the 2-min and 4-min groups. However, under this model, fearful responding should increase from CS+ onset until the time the US was expected (117 s post CS+ onset), and then decrease after this time regardless of whether or not the CS+ ceased to be presented prior to 117 s or continued to be presented past 117 s. This was not the case as seen in Figure 5b. For the 1-min group, US expectancy did not increase from 48 to 108 s post CS+ onset when the CS+ was removed from the screen. US expectancy did not decrease from 108 to 168 s post CS+ onset when the CS+ remained on the screen for the 4-min group. These findings are not consistent with the notion that the US is timed solely from the CS+ onset, regardless of subsequent CS+ presentation.

When considering re-test approximately 24 h after extinction, the present study found that although considerable cessation of responding had occurred, the CS+ was still more feared than the CS-, temporal knowledge of the US was retained, and extinction CS+ duration did not impact the degree of CS+ fear or temporal knowledge of the US at re-test. Thus, despite showing greater cessation of responding during extinction, the 1-min group did not outperform the other groups when extinction learning was re-tested 24 h after extinction on the original acquisition CS+ duration. RET would predict that between-session extinction would mainly be a function of total CS+ exposure as opposed to number of trials or temporal similarity to the acquisition CS+; such a pattern was observed in the present study. It should be noted that it is also possible that the absence of significant Group main or interactive effects at extinction re-test were due to lack of power to detect such effects.

In one way, the present extinction re-test findings are inconsistent with past animal studies which have found an extinction generalization decrement at re-test: the more temporally dissimilar the extinction duration from the acquisition duration, the less cessation of responding there is when re-tested at the acquisition duration (experiment 3, Drew et al., 2004; Plendl & Wotjak, 2010). It is possible that this generalization decrement at re-test was not observed in the present study because the magnitude of temporal difference between the acquisition and extinction durations was not large enough. Plendl and Wotjak used an

extinction duration ten times that of the acquisition duration and Drew and colleagues only observed a generalization decrement at re-test when the extinction duration was four times the acquisition duration. When Drew and colleagues extinction duration was only twice that of the acquisition duration (as in the present study), no generalization decrement was observed at re-test.

In another way, the present extinction re-test findings are consistent with past animal findings: extinction performance did not predict cessation of responding at re-test (Drew et al., 2004; Haselgrove & Pearce, 2003; Plendl & Wotjak, 2010). Because the experimental extinction phase can be viewed as an analogue of therapeutic exposures and re-test as an analogue of therapy follow-up, such findings have important implications for exposure therapy. Specifically, in contrast to theories that posit follow-up fear cessation to be a function of within-session extinction performance (e.g., Foa & Kozak, 1986), the present study provides evidence in support of Craske and colleagues' (2008) proposal that extinction performance is not equivalent to extinction learning.

For an equivalent amount of total CS+ exposure, all CS+ extinction durations resulted in equivalent re-test performance. This can be interpreted to mean that trials do not have to be as long, or longer, than acquisition duration to violate the temporal US expectancy and lead to fear cessation at re-test. Thus, total exposure time might be the key determinant to follow-up fear cessation meaning therapeutic exposures might not need to last as long, or longer, than the point at which an individual fears a negative outcome will occur to have longer-term extinction learning. However, it is important to consider that two other studies have shown that CS+ extinction durations that are significantly shorter, or longer, than those of acquisition result in poorer extinction at re-test (Drew et al., 2004; Plendl & Wotjak, 2010). Therefore, it is possible that exposure durations that are *too* dissimilar from the duration at which an individual fears the negative outcome will occur could result in poorer long-term outcomes.

A limitation of the present study is that CS+ extinction durations were only half as long, or twice as long as CS+ acquisition durations. It is possible that this difference in duration might not be large enough to detect generalization decrement effects. Future work with humans should use a greater range of CS+ extinction durations. Also, holding total CS+ exposure constant during extinction meant that there were different numbers of trials for different CS+ extinction durations. Future work should investigate the impact of CS+ duration on extinction and re-test performance when equating number of extinction CS+ trials, to determine how it compares to the present findings which equated for total CS+ exposure time. The 4-min group only had a single extinction exposure (due in part to equating total exposure rather than number of trials). Although extinction re-test for a single, long trial was no different than that for four shorter trials, it would also be beneficial to see the impact of multiple long trials on both extinction performance and re-test.

Another potential limitation is that the present study used CS durations that were considerably longer than those used in many studies of human fear conditioning (e.g., see Pineles, Orr, & Orr, 2009). The longer durations were chosen to allow for multiple SRs and expectancy ratings to be made during each CS and to allow enough time between ratings and SRs so that movement associated with ratings did not contaminate SR measurement. It is possible that the longer duration CSs resulted in participants experiencing a longer-lasting apprehensive state during the CSs as opposed to the state of fear generally assumed to accompany short duration CSs (for a discussion of anxiety versus fear, see Davis, Walker, Miles, and Grillon, 2010). Future work should examine if the present findings extend to the shorter duration CSs typically used in human fear conditioning. Another limitation of the present work is that although physiological indices were generally directionally consistent

with self-report, they were not always significant, and when they were their effect sizes were typically smaller than those of self-report. Perhaps the muscle stimulation was not aversive enough to result in a robust conditioned startle response as extent of fear responding is proportional to US magnitude (e.g., Morris & Bouton, 2006). Future work could potentially use a more aversive US.

The present study also used extinction ITI durations that differed from that of acquisition and re-test. The longer ITI was used to ensure that extinction CS+ trials were spaced far enough apart so participants would not view them as being part of a single, continuous presentation as posited to have occurred in past research (Cain, Blouin, & Barad, 2003). Extinction had a different temporal context than that of acquisition and re-test, which may have resulted in an ABA renewal effect (e.g., Bouton & Bolles, 1979). Thus, rather than being viewed as transfer of extinction learning to a later time, extinction re-test could be viewed as the extent to which renewal was prevented. Future work should use an ITI duration that is constant throughout acquisition, extinction, and re-test.

Despite these limitations, the present work is the first to demonstrate that humans develop a temporal expectation about when the US will be delivered that is specific to the CS+ and that this temporal expectation persists 24 h post-acquisition and 24 h post-extinction. Further, it demonstrated that for equal total CS+ exposure, cessation of fear responding at re-test is the same for CS+ extinction durations ranging from half the CS+ acquisition duration to twice the CS+ acquisition duration. Thus, temporal expectancy of the US in response to the CS+ can be violated by extinction durations different than that of acquisition. Finally, it also demonstrated that extinction performance does not predict extinction at re-test, which could have important implications for optimizing exposure therapy for anxiety disorders.

Highlights

Human differential fear conditioning was used to study (CS) temporal properties.
 Humans develop a temporal expectation about when the US will be delivered.
 Extinction performance did not predict extinction at re-test 24 h post extinction.
 Findings could have important implications for optimizing exposure therapy.

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Day 1	Day 2			Day 3
All Participants <u>Baseline</u> 2 'to be' CS+ (2 min each) 2 'to be' CS- (2 min each) 3 ITIs (1.5 min each) Pre-acquisition self-report <u>Acquisition</u> 4 CS+ (2 min each) 4 CS- (2 min each) 7 ITIs (1.5 min each) Post-acquisition self-report	1-min Group Pre-extinction self-report <u>Extinction</u> 4 CS+ (1 min each) 4 CS- (1 min each) 7 ITIs (10 min each) Day 2 time = 78 mins Post-extinction self-report	2-min Group Pre-extinction self-report <u>Pre- or post- extinction period</u> 40 min (4, 10-min 'ITI-like' blocks) <u>Extinction</u> 2 CS+ (2 min each) 2 CS- (2 min each) 3 ITIs (10 min each) Day 2 time = 78 mins Post-extinction self-report	4-min Group Pre-extinction self-report <u>Pre- or post- extinction period</u> 60 min (6, 10-min 'ITI-like' blocks) <u>Extinction</u> 1 CS+ (4 min each) 1 CS- (4 min each) 1 ITIs (10 min each) Day 2 time = 78 mins Post-extinction self-report	All Participants Re-test self-report <u>Re-test</u> 2 CS+ (2 min each) 2 CS- (2 min each)

Figure 1.

An overview of the experimental procedure with order of tasks listed from top to bottom within Day (e.g., for Day 3, participants first completed re-test self-report and then underwent the re-test phase). CS+ and CS- trials were presented in random order within phases. CS = conditional stimuli with only the CS+ being paired with the muscle stimulation, or unconditioned stimulus (US), during acquisition; ITI = intertrial interval, or the interval between the display of CSs when the movie is displayed onscreen; 'ITI-like' block = block of time equivalent to Day 2 ITI when movie was displayed onscreen and expectancy ratings and startle probes were presented at the same rate as during Day 2 ITIs; and Day 2 time refers to the amount of time each participant spent in the laboratory viewing either CSs or ITI movie during Day 2.

Figure 2.

Timing of US expectancy ratings and startle probes for a single conditional stimulus (CS) trial and the subsequent intertrial interval (ITI) for baseline, acquisition, and re-test as well as for each group (1-, 2-, and 4-min) during the extinction phase. Solid lines indicate time that CSs are displayed onscreen whereas dashed lines represent ITIs when the movie is displayed onscreen. Times presented are measured from CS onset during CS display and from CS offset (ITI onset) during ITIs. Distances between times is proportional to the amount of time passed with the exception of places where a jump in time is represented by two lines at a 45° angle.

Figure 3.

Acquisition dependent variable means and standard errors, collapsed across Group (1-, 2-, and 4-min) because there were no significant Group effects for acquisition. 3a: US expectancy ratings (0 = 'certain no stimulation', 5 = 'uncertain', and 10 = 'certain stimulation') were measured at two time points during CSs (early and late); measures within the same CS trial are connected by a line. 3b: Subjective fear (0 = 'not at all fearful of', 10 = 'very fearful of') was measured pre- and post-acquisition. 3c: Startle reflexes (SRs) were measured at two time points during CSs (early and late); measures within the same CS trial are connected by a line. SRs measured in microvolts were natural log transformed. 3d: Skin conductance responses (SCRs) measured in microsiemens (μ S) were only assessed once per CS trial (at onset).

Figure 4.

Extinction CS+ dependent variable means and standard errors presented separately by Group (1-, 2-, and 4-min). 4a: US expectancy ratings (0 = 'certain no stimulation', 5 = 'uncertain', and 10 = 'certain stimulation') provided within the same CS trial are connected by a line (e.g., for the 4-min Group all 4 US expectancy ratings are provided in response to a single CS+ trial, whereas for the 1-min group each rating is provided in response to a different CS+ trial). 4b: Subjective fear (0 = 'not at all fearful of', 10 = 'very fearful of') was measured pre- and post-extinction. 4c: Startle reflexes (SRs) measured within the same CS trial are connected by a line. SRs measured in microvolts were natural log transformed. 4d: Skin conductance responses (SCRs) measured in microsiemens (μ S) were only assessed once per CS trial (at onset).

Figure 5.

US expectancy rating (0 = 'certain no stimulation', 5 = 'uncertain', and 10 = 'certain stimulation') means and standard errors presented separately by Group (1-, 2-, and 4-min) for the CS+. Ratings provided within the same CS trial are connected by a line. Shading indicates the presence of the CS+ for the various groups as labeled; if the shading descriptor does not indicate that the CS+ is present for a particular group, then the CS+ is no longer onscreen for that group. 5a: Fourth and final CS+ acquisition trial. 5b: First CS+ extinction trial.

Figure 6.

Acquisition trial 4 and re-test dependent variable means and standard errors presented separately by Group (1-, 2-, and 4-min). 6a: US expectancy ratings (0 = 'certain no stimulation', 5 = 'uncertain', and 10 = 'certain stimulation') provided within the same CS trial are connected by a line. 6b: Subjective fear (0 = 'not at all fearful of', 10 = 'very fearful of') was measured post-acquisition and at re-test. 6c: Startle reflexes (SRs) measured within the same CS trial are connected by a line. SRs measured in microvolts were natural log transformed. 6d: Skin conductance responses (SCRs) measured in microsiemens (μ S) were only assessed once per CS trial (at onset).