Multiple fear-related stimuli enhance physiological arousal during extinction and reduce physiological arousal to novel stimuli and the threat conditioned stimulus

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Exposure therapy is a first line treatment of anxiety disorders, yet not all anxious individuals benefit in the short- or long-term highlighting a need for improvement. Whereas fears generalize easily to perceptually similar stimuli, fear extinction learning may not. Inclusion of multiple stimuli during extinction might enhance extinction retention, generalization of extinction learning to other novel stimuli, and fear reduction. Thirty-four unselected adults completed differential conditioning and extinction training in which one dog image conditional stimulus (CS+) was paired with an unconditional stimulus (US) (growl+scream), while a second dog image (CS-) was presented alone. During extinction, the Multiple group was exposed to unreinforced presentations of CS+ and CS- and two new dog images (i.e., M1; M2). The Control group was exposed to unreinforced CS+ and CS- matched on CS trial spacing to the Multiple group. During a generalization test, two new dog images were presented to both groups: GS Dog_Sim shared physical features with the CSs (encountered by both groups during extinction) and M2 (encountered only by the Multiple group during extinction), whereas GS Dog_Diff had distinctive physical features. During the extinction retest phase, the original CSs were presented unreinforced to both groups. During extinction, the Multiple group exhibited larger SCRs to both CSs compared to the Control group. During the generalization test, SCRs to GS Dog_Diff did not differ between groups, however, SCRs to GS Dog_Sim were smaller in the Multiple group than the Control group. SCRs were larger to GS Dog_Sim than GS Dog_Diff in the Control group whereas the inverse was found in the Multiple group. During the extinction retest, the Control group exhibited larger SCRs to the CS+ than to the CS- whereas there was no significant difference in the Multiple group. The Multiple group rated both CSs as more unpleasant compared to the Control group after extinction, the generalization test phase and the extinction retest phase. Exposure to multiple stimuli enhanced generalized physiological arousal during extinction, yet reduced physiological arousal during subsequent exposure to novel stimuli and re-exposure to the CS+. Negative evaluations of both CSs seemed resistant to extinction with multiple feared stimuli, however, post-phase CS ratings may invoke recall of enhanced arousal during extinction and trial-by-trial CS evaluations should be assessed. Results suggest that multiple stimuli during exposure therapy may reduce physiological arousal to novel stimuli and the original feared stimulus after treatment.

Keywords: Extinction; multiple stimuli; generalization, anxiety

Introduction

Anxiety disorders are among the most common and debilitating disorders with prevalence rates suggesting between 28-33% of people are likely to experience an anxiety disorder during their lifetime (Baxter et al., 2013; Kessler et al., 2005, 2012). Anxiety disorders are highly comorbid and when untreated can lead to significant impairment (Goetzel, Hawkins, Ozminkowski, & Shaohung, 2003; Kessler et al., 1997; 2012). Exposure-based cognitive-behavioural therapy is a first line psychological treatment for anxiety disorders (James, James, Cowdrey, Soler, & Choke, 2015; Saavedra, Silverman, Morgan-Lopez, & Kurtines, 2010). Exposure therapy involves repeated and prolonged exposure to a feared stimulus in order to violate outcome expectancies, eliminate negative evaluations, and reduce fear (Craske et al., 2008; 2014). However, positive treatment-outcome rates hover around 60% and approximately half of those individuals who experience a successful post-treatment outcome are likely to relapse over time (Ginsburg et al., 2014; Loerinc et al., 2015). These findings highlight the need for further research to improve short- and long-term treatment outcomes.

Learning theories provide the dominant framework for understanding the development and treatment of anxiety disorders. They propose that anxiety develops via a number of learning-related pathways one of which is classical conditioning (Rachman, 1977). A fear response is induced in classical conditioning by pairing a neutral conditional stimulus (CS+; e.g., a shape) with an aversive unconditioned stimulus (US; e.g., a scream). Initially the CS does not elicit an emotional reaction. However, after repeated pairings of the CS and the US, this CS will elicit a conditioned response (CR), which may be characterised by increases in self-reported anxiety or in physiological responses such as skin conductance responses, relative to a control stimulus that was presented alone (CS-; e.g., Waters, Henry & Neumann, 2009).

Research examining fear learning in anxious and non-anxious individuals suggests that pathological anxiety is associated with enhanced responding to the CS+ in single cue paradigms as well as a tendency to generalize fear responding to stimuli similar to the conditioned fear cue, including the CS-(e.g., Duits et al., 2015; Lissek et al., 2005; Pearce, 1987). For example, stimuli that share physical characteristics with the CS+ can evoke a certain extent of conditioned responding (e.g., Lissek et al., 2008; Vervliet, Vansteenwegen, Baeyens, Hermans, & Eelen, 2005; Vervliet, Kindt, Vansteenwegen, & Hermans, 2010; Vervliet, Vansteenwegen, & Eelen, 2004). This generalization of fear can complicate psychological treatments. Extinction-based treatments involve repeated exposures to fear-evoking stimuli (i.e., the CS+) until fear declines (Lissek et al., 2008). Exposure techniques are highly efficacious but previous research has shown that whereas acquisition of conditioned fear generalizes easily over perceptually similar stimuli, extinction of fear may not (e.g., Vervliet et al., 2005; Vervliet et al., 2010).

Numerous studies have examined the generalization of fear extinction learning to other stimuli by including stimuli that are perceptually and/or conceptually similar to the CS+ during extinction, i.e., generalization stimuli (GSs) (e.g., Lissek et al., 2008; Pappens, Schroijen, Van den Bergh, & Van Diest, 2015; Vervliet et al., 2005; 2010; Vervoort, Vervliet, Bennett, & Baeyens, 2014). For example, following an acquisition phase involving one shape (CS+) paired with shock and a second shape (CS-) presented alone, participants assigned to the extinction control group received four presentations each of the original CS+ and CS- without the US. The generalization group received four presentations of each of two GSs (GS1; GS2, without the US) and no presentations of the original CS+ and CS- (Vervliet et al., 2005). Generalization stimuli were categorically and perceptually similar to the CS+ and CS- (i.e., shapes). No differences were found between the groups during extinction. However, during test with the original CSs, the generalization group showed increased

responding to the CS+ compared to the CS- which was not observed in the extinction control group. Similar results have been found in studies of categorical fear extinction generalization, whereby fear to the original CS+ did not decline following extinction with stimuli that were categorically similar to the CS+ (e.g., Vervoort et al., 2014). Thus, when the CS+ *itself* is extinguished, extinction learning appears to persist with no differences between CSs observed at test. However, repeated presentation of stimuli that are perceptually or conceptually *similar* to the CS+ during extinction (in absence of the CS+) does not significantly reduce fear of the CS+. These differences have important practical implications given that exposure therapy is almost always conducted with generalization stimuli and not the original CS+.

It is also noteworthy that these studies assessed the generalization of fear extinction in test phases that included only the original CSs, but not novel stimuli that were distinct from the original CSs and GSs used during extinction (e.g., Pappens et al., 2015; Vervliet et al., 2005; 2010). Other studies that have examined fear extinction generalization to novel stimuli have found mixed evidence. Some have documented smaller responses to the CS+ and increasingly larger responses to GSs of increasing dissimilarity (e.g., Bass & Hull, 1934; Hovland, 1937; Myers & Davis, 2007) whereas other studies have found no evidence of extinction generalization as a function of stimulus similarity (e.g., Pappens et al., 2015). Furthermore, clinical analogue studies that compared responding to novel stimuli and the original CSs after exposure to either multiple feared stimuli (akin to multiple GSs) or the same feared stimulus (akin to a single GS given the original CS+ was not included in either condition) have found increased physiological and emotional reactivity during extinction, enhanced extinction generalization (i.e., less fear responding to novel stimuli post-extinction), and enhanced extinction retention (i.e., less fear responding to the original test stimulus) in the multiple stimulus group. For example, Rowe and Craske (1998) found more fear across exposure trials and a trend towards higher anxiety post-treatment in response to the original

test spider, but also less fear to a novel spider in spider phobic adults exposed to four different spider stimuli during extinction compared to repeated exposure to the same spider. Similarly, Shiban, Schelhorn, Pauli, and Mühlberger (2015) found that exposure to multiple compared to a single spider stimulus produced stronger short- and long-term fear reductions.

It has been proposed that exposure to multiple feared stimuli during extinction/ exposure therapy might enhance variability in emotional responding and sustain arousal and engagement during exposure sessions which may enhance extinction learning (e.g., Craske et al., 2014; Waters, Potter, Jamesion, Bradley, & Mogg, 2015). The precise mechanism(s) underlying increased reactivity during extinction/exposure therapy are unclear. Presenting multiple feared stimuli during extinction might increase arousal by facilitating attention and elaborative stimulus processing. This, in turn, may enhance learning that a wide array of stimuli that are directly (CS+) and indirectly (GSs due to CS+ similarity but no direct conditioning) associated with threat (i.e., the US) are associated with safety (i.e., US absence; Waters & Craske, 2016). Thus, presenting multiple and varied stimuli might be one avenue for making learning during extinction more salient and memorable (cf. Bjork & Bjork, 1992), thereby strengthening the likelihood of retrieval of extinction memories and reduced fear upon subsequent exposure to novel stimuli (i.e., generalization test) or the original CS+ (i.e., extinction retest).

The present study aimed to determine the effects of conducting extinction trials with multiple stimuli (the original CSs and novel GSs) relative to extinction with the original CSs only on the generalization of extinction learning to novel stimuli and reactivity upon re-exposure to the original CSs. We tested the hypothesis that extinction training with multiple stimuli (i.e., CSs and GSs; Multiple condition) relative to extinction-as-usual with the original CSs only (Control condition) would (a) enhance physiological arousal (as indexed by skin conductance responses; SCRs) and emotional reactivity (as indexed by subjective

anxiety ratings and CS evaluations) during extinction, and in turn, (b) result in lower physiological arousal and emotional reactivity to a new GS that is perceptually more similar to the original CSs and the extinction stimuli relative to a new GS that is perceptually more distinct to the extinction stimuli, and (c) result in lower physiological arousal and emotional reactivity to the original CS+ at extinction retest. To enhance ecological validity and because abstract shape stimuli evoke low level processing in comparison to real life stimuli (Dunsmoor & Murphy, 2015) we utilised dog images as the CSs and GSs and an aversive growl coupled with a scream as the US.

Method

Participants

Participants were 40 undergraduate psychology students between 18 and 42 years of age (M = 23.5 years, SD = 7.7) who participated in exchange for partial course credit. Participants were randomly assigned to one of two groups: Multiple (N = 20) and Control (N = 20). Of these 40 participants, five were excluded due to unscorable skin conductance data and one participant was excluded due to restless behaviour during the acquisition phase and failure to demonstrate CS-US contingency awareness. Of the final sample, the Multiple group consisted of 18 participants (14 female, 4 males) and the Control group consisted of 16 participants (12 females, 4 males). Sample size was based on prior Pavlovian conditioning and extinction studies involving between groups comparisons (e.g., Waters, Henry, & Neumann, 2009; Howley & Waters, 2017; Waters & Kershaw, 2015; Waters, Theresiana, Zimmer-Gembeck, & Craske, 2017; Waters, 2017).

Materials and Measures

Anxiety symptoms. The State-Trait Anxiety Inventory for Adults (STAI; Spielberger, Gorsuch, Lushene, Vagg & Jacobs, 1983) was used to assess anxiety symptomology. The STAI is a 40-item self-report scale comprised of two 20-item scales designed to differentiate between

the temporary condition of "state anxiety" (State scale) and the more general and long-standing quality of "trait anxiety" (Trait scale). Each subscale score ranges from 20 to 80, with higher scores indicating higher anxiety.

Fear of dogs. The Dog Phobia Questionnaire (DPQ; Hong & Zinbarg, 1999) was used to assess fear of dogs to ensure a normative sample. The DPQ is a 27-item self-report questionnaire with a 7-point response scale designed to measures symptoms of dog phobia. Scores range from 27 to 189 with higher scores indicating more fear of dogs.

Stimuli. The US was an unpleasant 3 sec sound of a dog growling and a woman screaming set at 100 dBA and delivered through Sony stereophonic headphones. The US was presented binaurally through the headphones with the growl commencing .5 s before the scream and then both sounds presented simultaneously for the remaining 2.5 s. The CSs were photographs of dogs (see Figure 1). Two pictures were allocated as CSs counterbalanced across participants. Photographs of four additional dogs were used; two as the additional stimuli during extinction for the Multiple group (M1; M2) and two as generalization stimuli in the generalization test (GS Dog_Sim; GS Dog_Diff). All dog images were the same size, presented in colour and set against a white background. All dogs were standing side on and looking forward. Dogs differed in breed (e.g. Boxer, German Shepherd, Mastiff, Doberman, Great Dane; Rottweiler). One generalization dog, GS Dog_Sim, had features that were perceptually similar to the CSs (e.g., shape; size; orientation; therefore similar to dogs encountered by the Multiple and Control group) as well as the GSs in terms of the black coat colour (e.g., M2; therefore similar to a dog encountered only by the Multiple group). The second generalization dog, GS Dog_Diff was perceptually more distinct from the CSs and GSs used during extinction with a black and white spotted coat (and thus different to stimuli encountered by both groups; see Figure 1). The images were presented for 8s in the centre of

the screen on a Dell 19" colour monitor at a distance of approximately 80 cm and a visual angle that averaged 9.6 degrees.

Insert Figure 1

Skin conductance responses (SCR). Skin conductance was recorded using pre-gelled isotonic electrodes placed on the palm of the participant's non-dominant hand. It was acquired using a Biopac data acquisition system (Model MP150) with a sampling frequency of 2000 Hz via a EDA100C amplifier. Data were analysed using Acqknowledge software Version 4.4.0. Respiration was recorded using a Biopac TSD201 transducer connected to an RSP100C transducer amplifier, to monitor for respiratory influences on SCRs.

CS valence and arousal ratings. Participants rated the arousal and valence of the CS+ and CS- dog images prior to acquisition, post-acquisition, post-extinction, and following generalization and extinction retests. Arousal was rated using a Likert scale ranging from 1 (calm) to 9 (very aroused), whereas valence was rated using a two tailed Likert scale ranging from 1 (very pleasant) to 5 (neutral) to 9 (very unpleasant).

Subjective anxiety. Participants rated their subjective level of anxiety prior to acquisition, post-acquisition, post-extinction, and following generalization and extinction retests, using a one tailed Likert scale ranging from 0 (not at all) to 10 (very anxious).

Contingency awareness. Upon completion of the acquisition phase, participants were asked whether they noticed if the sound delivered through the headphones coincided with any of the stimuli presented on the screen. If the participant responded with "Yes", they were asked to identify which dog was presented with the sound and responses were recorded verbatim. Participants were considered contingency aware if they identified that the sound was paired with the CS+.

Procedure

Ethics approval was obtained from Griffith University Research Ethics Committee. Participants were provided with an information sheet and gave written consent. Each assessment session lasted approximately 1.5 hours and took place within two weeks of the student expressing interest to participate.

After introduction to the laboratory, two electrodes were attached to the palm of the participant's non-dominant hand to record SCRs. A respiration belt was placed around the chest area to monitor respiration and control for artefacts in the SCR data. Participants were seated alone in an experimental room within the laboratory and connected by a closed circuit camera to a control room.

Acquisition phase. Participants rated their subjective level of anxiety and the valence and arousal of the dog images using the SAM. Next, they were informed that they would be presented with images on the computer screen, one after the other, and that they would hear a loud sound presented through the headphones from time to time. The headphones were fitted, the researcher exited the room, and participants received instructions on the computer screen that they would see images and hear sounds and were asked to pay attention throughout the experiment.

Next, participants were presented with a random sequence of 12 CS+ and 12 CS- trials (see Figure 2) with the caveats that the first two trials were a CS+ and a CS- (counterbalanced across participants) and that subsequently no more than two trials of either CS were presented consecutively. A fixation cross was presented in the centre of the screen during inter CS intervals which was replaced by the 8 s CSs. The US was presented during the last 3 s of the CS+. The intertrial interval varied between 25 – 30 s. After the 24 trials, the researcher entered the room and removed the participant's headphones. Participants rated their subjective anxiety and the valence and arousal of the CSs and contingency awareness

was assessed. Then participants were informed that the task would continue, headphones were fitted, and the researcher left the room.

***Extinction phase*.** Participants in the Control Group were presented with a random sequence of 12 CS+ trials (without the US) and 12 CS- trials with the caveat that the first two trials were a CS+ and a CS- trial (counterbalanced across participants) and that no more than two consecutive trials were the same. Participants viewed a fixation cross during the intertrial interval of 48-52 s. Participants in the Multiple Group were presented with the same sequence of CS+ CS- trials as well as with 12 trials of each of two GSs. GSs were presented one at a time during the inter CS interval starting 20-22 s after CS offset . Groups were matched for the number of CSs seen during extinction, CS trial spacing, and phase duration (see Figure 2).

After the extinction trials, the researcher entered the room and removed the headphones. Participants rated subjective anxiety and valence and arousal of the CSs using the SAM. Participants were informed that the task would continue, headphones were fitted and the researcher left the room.

***Generalization test phase**:* Participants in both groups were presented three times with two novel dogs (GS Dog_Diff; GS Dog_Sim) in random order (see Figures 1 and 2)[1]. The generalization test was followed by a rating of participant anxiety and CS valence and arousal.

Extinction retest phase: During the extinction retest, both groups were presented with four CS+ and four CS- trials in random order (see Figure 2) followed by a final rating of participants' anxiety, and CS valence and arousal. Participants were debriefed and awarded course credit.

Insert Figure 2

Response Definitions and Data analysis

Skin conductance responses. Participants were observed throughout the experiment and movement, excessive drowsiness and behaviours such as coughing and sneezing were recorded (less than 2.7% of responses). Trials in which such behaviours occurred were rejected.

The magnitude of the SCR elicited during the presentation of each CS and GS was scored within two latency windows: first interval responses (FIR) and last interval responses (LIR). Scoring SCRs within multiple latency windows has been shown to have numerous advantages over entire interval scoring (Luck & Lipp, 2016). Each SCR was scored as the difference between the trough and apex of the curve and expressed in microsiemens (μS). First interval responses (FIR) commenced within 1-5 s after stimulus onset, reflecting the initial orienting response (see Öhman, 1983; Öhman & Bohlin, 1973; Prokasy, 1977). Late interval responses (LIR) commencing within 6-12 s after CS onset reflect the response to the 3 s US on CS+ trials (which onset 5 s after CS+ onset) or US absence on CS- trials and extinction trials (Prokasy, 1977; Prokasy & Kumpfer, 1973). Skin conductance responses were square root transformed in order to normalise the distribution (Venables & Christie, 1980).

Analyses were conducted separately for FIRs and LIRs for the acquisition and extinction phases using 2 (Group: Multiple; Control) × 2 (CS: CS+, CS-) × 4 (Block: 1-4 averaging across three trials per block) linear mixed models for repeated measurements with Satterthwaite's approximation for degrees of freedom. An additional analysis compared FIRs to the CSs and GSs within the Multiple group using a 4 (Stimulus: CS+, CS-, M1, M2) x 4 (Block: 1-4) linear mixed model.

FIRs and LIRs for the generalization test phase were analysed in a 2 (Group: Multiple; Control) × 2 (GS: GS Dog_Diff; GS Dog_Sim) × 3 (Trial Number: 1 – 3) linear

mixed models analysis of variance (ANOVA) for repeated measurements with Satterthwaite's approximation for degrees of freedom applied.

FIRs and LIRs for the extinction retest phase were subjected to 2 (Group: Multiple; Control) × 2 (CS: CS+; CS-) × 4 (Trial Number: 1 – 4) linear mixed models analysis of variance (ANOVA) for repeated measurements with Satterthwaite's approximation for degrees of freedom applied.

Subjective ratings. Participants' CS evaluations and anxiety ratings (using an 11-point scale) were analysed using 2 (Group: Multiple; Control) × 5 (Phase: Pre-acquisition, Post-acquisition, Post-extinction; Post-generalization test; Post-extinction retest) mixed model factorial ANOVAs. All follow-up comparisons were Bonferroni corrected to control for the accumulation of error due to multiple comparisons.

Results

Control analyses

There were no significant differences between groups in age, (Control *M*: = 23.05 (SD = 8.36); Multiple *M*: = 23.56 (*SD* = 7.98), gender (Control: 77% female; Multiple: 72% female), DPQ scores (Control *M*: = 69.31, *SD* = 24.17; Multiple *M:* = 62.11, *SD* = 23.46), and STAI-Trait scores (Control *M*: = 45.06, *SD* = 10.33; Multiple *M:* = 42.00, *SD* = 11.47), all *F*'s < 2.01, *p*'s > .10.

Skin Conductance Responses

Acquisition phase

FIR. As shown in Figure 3a, the CS+ elicited larger FIRs than the CS- throughout acquisition, significant main effect of CS, $F(1, 395.07) = 8.40$, $p = .004$. A significant main effect for Block also emerged, $F(1, 178.29) = 2.69$, $p = .046$, but follow-up analyses were not significant after Bonferroni correction (all $p > .06$).

LIR. As shown in Figure 3b, LIRs differed as a function of Group, $F(1, 197.35) = 9.80$, $p = .002$, CS, $F(1, 348.04) = 193.04$, $p < .001$, and Block, $F(3, 301.55) = 9.26$, $p < .001$. Significant interactions were found between Group and CS, $F(1, 348.04) = 5.56$, $p = .019$, and CS and Block, $F(3, 382.62) = 22.56$, $p < .001$.

Follow-up comparisons of the CS x Block interaction revealed a significant main effect of Block for the CS+, $F(3, 280.87) = 2.70$, $p = < .046$. LIRs to the CS+ were significantly larger in Block 1 compared to Block 2 ($p = .001$); subsequent differences were not significant (all *p's* > .10). There were no significant differences in LIRs to the CS-, $F(3, 273.04) = 1.10$, $p = .35$.

Follow-up comparisons of the CS x Group interaction revealed no significant group differences for the CS+, $F(1, 77.37) = 0.82$, $p = .37$. LIRs to the CS- were unexpectedly larger in the Multiple than the Control group, $F(1, 95.15) = 10.57$, $p = .002$[2].

Insert Figure 3

Extinction phase

FIR. As shown in Figure 3c, analysis of FIRs revealed a significant main effect of CS, $F(1, 337.38) = 7.26$, $p = .007$ reflecting significantly larger FIRs to the CS+ compared to the CS-. A significant main effect of Group was also found, $F(1, 218.62) = 5.13$, $p = .02$, as well as a Group x Block interaction, $F(3, 314.73) = 2.85$, $p = .037$. This reflected that collapsed across CSs, FIRs were significantly larger in the Multiple group compared to the Control group during Block 2 and Block 3 (both $p < .02$) but not in Block 1 and Block 4 (both $p > .72$).

The additional analysis comparing FIRs to the CSs and GSs within the Multiple group revealed no significant results, all *F's* < 1.55, *p*'s > .12 (see Figure 4). Thus, the Multiple group exhibited larger SCRs to both CSs than the Control group during the middle blocks of extinction.

LIR. As shown in Figure 3d, analysis of LIRs revealed a significant main effect of Group, $F(1, 193.71) = 13.05$, $p < .001$, and a significant interaction between Group and CS, $F(1, 349.67) = 9.63$, $p = .002$.

Follow-up comparisons of the Group x CS interaction revealed significantly larger LIRs to the CS+ in the Multiple Group than the Control Group $F(1, 115.85) = 24.84$, $p < .001$, with no significant differences for the CS-, $F(1, 124.96) = 1.80$, $p = .18$. Moreover, contrasting the CSs within each group revealed significantly larger LIRs to the CS- than the CS+ in the Control group, $F(1, 191.28) = 8.88$, $p = .003$, with no significant differences in the Multiple group, $F(1, 172.60) = 3.09$, $p = .08$.

An additional analysis comparing LIRs to the CSs and GSs in the Multiple group revealed no significant differences, all F's < 1.60, p's $> .13$. (see Figure 4).

Insert Figure 4

Generalization test phase

FIR. As shown in Figure 3e, FIRs differed significantly across trials, $F(2, 144.24) = 7.50$, $p < .001$, and a significant interaction between Group and GS emerged, $F(1, 86.95) = 16.48$, $p < .001$.

For the Multiple group, FIRs were significantly smaller to GS Dog_Sim than to GS Dog_Diff ($p = .009$) whereas for the Control group, FIRs were significantly larger to GS Dog_Sim compared to GS Dog_Diff ($p = .003$). Furthermore, the Control groups' FIRs were significantly larger to GS Dog_Sim compared to the Multiple group ($p = .006$) whereas the groups did not differ significantly in FIRs to GS Dog_Diff ($p = .68$). Thus, the Multiple group exhibited less physiological arousal to the generalization stimulus that was the most similar to the extinction stimuli whereas the Control group exhibited the inverse pattern.

LIR. The analysis of LIRs revealed no significant differences (all $p > .14$) (see Figure 3f).

Extinction retest phase

FIR. As shown in Figure 3g, the analysis of FIRs revealed no significant main or interaction effects (p's > .12).

LIR. The analysis of LIRs revealed a significant Group x CS interaction, $F(1, 126.97) = 6.49$, $p = .01$ (see Figure 3h). LIRs to the CS+ were significantly larger than to the CS- in the Control group, $F(1, 349.67) = 4.13$, $p = .03$, but not in the Multiple group, $F(1, 56.35) = 0.09$, $p = .76$. Differences in LIRs between groups at each level of CS were not significant (both p's > .07). Thus, the Control group exhibited larger LIRs to the CS+ than the CS- upon re-exposure to the original CSs.

Subjective measures

CS Arousal. Analysis of participants' CS arousal ratings (see Figure 5, upper panel) yielded significant main effects of Phase, $F(4, 29) = 9.12$, $p < .001$, $\eta_p^2 = .56$, and CS, $F(1, 32) = 36.01$, $p < .001$, $\eta_p^2 = .53$, and a significant interaction between Phase and CS, $F(4, 29) = 9.02$, $p < .001$, $\eta_p^2 = .55$.

Insert Figure 5

Follow up pairwise comparisons of the Phase x CS interaction revealed a significant effect of Phase for the CS+, $F(4, 30) = 10.82$, $p < .001$, $\eta_p^2 = .59$. This reflected that arousal ratings of the CS+ increased significantly from pre-acquisition to post-acquisition ($t(33) = 5.96$, $p < .001$, $d = 0.96$), and decreased from post-acquisition to post-extinction ($t(33) = 6.11$, $p < .001$, $d = 0.90$). No significant differences were found between subsequent phases (t's < .62, $p > .56$, $d < .09$). A significant effect of Phase was also found for the CS-, $F(4, 30) = 5.61$, $p = .002$, $\eta_p^2 = .43$. However, this reflected that the CS- was rated more arousing at pre-acquisition than post-acquisition ($t(33) = 3.74$, $p < .001$, $d = 1.39$) with no differences at subsequent phases (t's < .83, $p > .66$, $d < .10$). Furthermore, the CS+ was rated more arousing than the CS- at post-acquisition ($t(33) = 7.04$, $p < .001$, $d = 1.32$), post-extinction

($t(33) = 2.79$, $p = .009$, $d = 0.49$), post-generalization ($t(33) = 3.19$, $p = .003$, $d = 0.55$), and at post-retest ($t(33) = 3.19$, $p = .003$, $d = 0.57$), but not at pre-acquisition ($t(33) = 1.85$, $p = .08$, $d = 0.30$).

CS Valence. Analysis of participants' CS valence ratings (see Figure 5, lower panel) yielded significant main effects of Phase, $F(4, 29) = 3.86$, $p = .012$, $\eta_p^2 = .35$, and CS, $F(1, 32) = 13.91$, $p = .001$, $\eta_p^2 = .30$, and significant interactions between Phase and Group, $F(4, 29) = 2.91$, $p = .04$, $\eta_p^2 = .28$, and Phase and CS, $F(4, 29) = 5.55$, $p = .002$, $\eta_p^2 = .43$.

Follow-up comparisons of the Phase x CS interaction revealed a significant effect of Phase for the CS+, $F(4, 30) = 7.02$, $p = <.001$, $\eta_p^2 = .48$. This reflected that the CS+ was rated significantly more unpleasant from pre-acquisition to post-acquisition ($t(33) = 4.42$, $p < .001$, $d = 0.70$) and more pleasant from post-acquisition to post-extinction ($t(33) = 2.90$, $p = .007$, $d = 0.43$). No significant differences were found between subsequent phases (t's $< .67$, $p > .58$, $d < .10$). Conversely, the effect of Phase was not significant for the CS-, $F(4, 30) = 1.72$, $p = .17$, $\eta_p^2 = .19$. Furthermore, the CS+ was rated as significantly more unpleasant than the CS- at post-acquisition ($t(33) = 5.11$, $p < .001$, $d = 1.01$), post-extinction ($t(33) = 2.51$, $p = .017$, $d = 0.31$), post-generalization ($t(33) = 2.44$, $p = .02$, $d = 0.33$), and at post-extinction retest ($t(33) = 2.55$, $p = .016$, $d = 0.32$), but not at pre-acquisition, ($t(33) = 0.78$, $p = .44$, $d = 0.16$).

Follow up comparisons of the Phase x Group interaction revealed a significant effect of Phase for the Control group $F(4, 12) = 7.51$, $p = .003$, $\eta_p^2 = .71$. This reflected that the Control group rated the CSs as significantly more pleasant at post-extinction compared to post-acquisition ($t(33) = 4.42$, $p < .001$, $d = 0.70$). No significant differences were found between subsequent phases (t's $< .81$, $p > .62$, $d < .11$). Difference in CS ratings between phases were not significant in the Multiple group $F(4, 14) = 5.53$, $p = .07$, $\eta_p^2 = .15$. However, the Multiple Group rated both CSs as significantly more unpleasant than the

Control group at post-extinction ($t(32) = 2.17$, $p = .038$, $d = 0.74$), post-generalization test ($t(32) = 2.13$, $p = .04$, $d = 0.73$), and post-extinction retest ($t(33) = 2.44$, $p = .02$, $d = 0.84$).

Subjective anxiety. The ANOVA of subjective anxiety ratings revealed a significant main effect of Phase, $F(4, 29) = 16.20$, $p = < .001$, $\eta_p^2 = 0.69$ (see Figure 6). Subjective anxiety ratings significantly increased from pre- to post-acquisition ($t(33) = 2.80$, $p = .009$, $d = 0.09$) and then declined significantly from post-acquisition to post-extinction ($t(33) = 7.15$, $p < .001$, $d = 1.03$) and from post-extinction to post-generalization test ($t(33) = 3.19$, $p = .003$, $d = 0.13$) with no significant differences between post-generalization and post-extinction retest ($t(33) = 1.78$, $p = .08$, $d = 0.04$). Although suggestive, the Group by Phase interaction was not significant, $F(4, 29) = 1.87$, $p = .14$, $\eta_p^2 = 0.21$. No other effects were significant (all F's < 1.01, $p > .49$).

Insert Figure 6

Discussion

Several key findings emerged from this study. Partially consistent with hypotheses, participants exposed to multiple dog images during extinction exhibited larger first interval SCRs to both CSs than the control group, and larger last interval SCRs to the CS+ compared to the CS-, a difference not found in the control group. Furthermore, during the generalization test, the multiple group exhibited smaller first interval SCRs to the similar generalization dog compared to the different generalization dog and the control group. In contrast, the Control group exhibited larger first interval SCRs to the similar generalization dog compared to the different generalization dog. However, there were no significant group differences in SCRs to the different generalization dog. As expected, participants in the multiple group did not display differential SCRs to the CS+ and CS- during the extinction retest phase whereas the control group exhibited significantly larger last interval SCRs to the CS+ compared to the CS-. Finally, partially consistent with hypotheses, the multiple group gave more negative

ratings of both CSs after extinction than did controls and continued to rate both CSs as significantly more negative compared to controls after the generalization test and extinction retest. There were no group differences in ratings of CS arousal and subjective anxiety.

The present findings are consistent with previous evidence of increased arousal during and higher anxiety after exposures to multiple stimuli that include the original CSs and stimuli that are categorically similar to, but perceptually different from the CS+, as well as stronger extinction generalization and retention (e.g., Rowe & Craske, 1998; Shiban et al., 2015). Together, results suggest that exposure to multiple stimuli enhances physiological arousal during and negative CS evaluations after extinction, yet subsequently reduces physiological arousal to new, perceptually similar stimuli and the original CS+.

Presenting multiple stimuli during exposure may increase physiological arousal by invoking greater attentional engagement and elaborative processing of stimuli that are directly (CS+) and indirectly (CS-; generalization stimuli) associated with threat in order to differentiate between stimuli and establish new CS – no US associations (Rescorla & Wagner, 1972; Waters & Craske, 2016). Greater engagement via enhanced processing of stimulus features and contingencies during extinction may in turn enhance the salience of extinction memories, thereby strengthening the likelihood of their recall when subsequently exposed to novel generalization stimuli or the original CSs after extinction (cf. Bjork & Bjork, 1992; Hoyland, 1973).

However, the pattern of results observed in the generalization test suggests that the application of extinction learning to new stimuli depended upon the degree of perceptual similarity between the extinction and generalization test stimuli (cf. Hoyland, 1973). Notably, groups did not differ in response to the spotted dog which was the most dissimilar generalization stimulus relative to the CSs and the additional dogs (M1; M2) used during extinction. Rather, they differed in response to the stimulus that was the most similar to the

stimuli presented during extinction. Responding in the multiple group to the similar generalization stimulus may have been reduced compared to the dissimilar generalization stimulus because it shared physical features with the CSs and M1 and M2 dogs used during extinction. Conversely, larger responding to the similar than the dissimilar generalization stimulus in the control group suggests that extinction learning did not generalize to novel dog stimuli after exposure to CS+ and CS- only during extinction. Instead, the pattern of responding for the control group suggests that properties of the novel generalization test dogs themselves influenced responding, whereby the black dog may have been more distinctive and arousing than the spotted dog. Together, findings suggest that the extent of prior exposure to a variety of stimuli with varying physical features modulates physiological reactivity to novel stimuli of varying degrees of perceptual distinctiveness; broader exposure reduces reactivity along a stimulus similarity continuum whereas narrow exposure does not and instead may enhance reactivity based on features of the novel stimuli themselves. Future studies should examine physiological arousal reductions to novel stimuli with the inclusion of an even wider variety of perceptually distinct stimuli during extinction.

Although the multiple group was hypothesized to rate the CS+ as more negative after extinction than the control group, larger negative evaluations of both CSs persisted after the post-generalization test and post-extinction retest. Prior research has found that CS valence is more resistant to extinction than physiological responses, especially for fear-relevant stimuli, although this is typically observed to be specific to the CS+ only (Luck & Lipp, 2015; 2016). As CS evaluations were assessed after each phase in the present study rather than trial-by-trial during each phase, it is also plausible that the brief separation and distinctiveness of CS evaluation assessments from the preceding phase triggered memories of elevated arousal and engagement during extinction rather than the preceding test phases. The assessment of both

trial-by-trial and between-phase ratings would help elucidate the nature of CS evaluations during and after extinction training with multiple stimuli.

Contrary to hypotheses and findings from previous studies (e.g., Rowe & Craske, 2008; Shiban et al., 2015), there were no significant differences between groups in subjective ratings of anxiety across phases. These non-significant outcomes may be due to the low levels of anxiety in the non-clinical sample of the present study and suggest that studies with clinical samples are warranted (e.g., Shiban et al., 2015). In addition, it is possible that the US was not sufficiently aversive to elicit anxiety-related differences. Future studies should also utilize a more aversive US such as shock.

Although we had not formulated hypotheses in relation to responses to the CSs versus the additional stimuli (M1; M2) during extinction, one might expect that the novelty of the latter stimuli might invoke larger SCRs compared to the CSs. In the present design, the first presentations of M1 and M2 were always preceded by an unreinforced presentation of either the CS+ or CS- which themselves may have attracted attention and evaluative processing to establish US absence, thereby elevating physiological arousal prior to the presentations of M1 and M2. Furthermore, unlike the CS+ and the CS- during acquisition, the additional stimuli had never been presented in the same phase with the US. Therefore, the associative strength of M1 and M2, and therefore the extent of reactivity towards them, may not have been greater than that of the CSs. Further studies that vary the presentation order of the CSs and additional stimuli would clarify these possibilities.

Other study limitations should be considered. Although we assessed participants' level of fear of dogs, a limitation of the current study was that participants' prior experience and familiarity with dogs was not taken into account, for instance, it is not known whether participants had dogs as pets. Furthermore, although using colour picture stimuli enhances ecological validity, dog images may be inherently more fear provoking than other CSs (e.g.,

shapes) which could have affected the results (cf. Luck & Lipp, 2016). Also, participants' subjective perceptions of the similarity of the dog stimuli employed in the present study were not assessed to avoid drawing attention to similar/different features of the dogs. However, this should be examined in a separate study in the future. Although the two groups were matched on CS trial spacing, number of CS exposures, and duration of the extinction phase, it is possible that results in the Multiple group were due to exposure to a larger number of stimuli than presented in the Control group. Future studies should include a second control condition which equates the number of stimulus presentations. The present study also does not elucidate the precise mechanism underlying increased physiological arousal during extinction with multiple stimuli. Future research should monitor eye movements to assess visual attention allocation and trial-by-trial stimulus evaluations in addition to between-phase ratings. Finally, a longer-term follow-up assessment was not included which limits conclusions about the stability of the group differences over time. Future studies should include a follow-up assessment one to two weeks later (e.g., Rowe & Craske, 1998).

The present findings also have several practical applications. They suggest that multiple stimuli from the same category as the original feared stimulus should be included during exposure therapy in order to enhance the generalization of extinction learning to stimuli beyond those used during exposure therapy. Moreover, the present results suggest that generalization effects may be further enhanced by the inclusion of stimuli from the same category that are as perceptually diverse as possible. To some extent, these principles underpin intensive forms of exposure therapy, such as one session treatment (OST) of specific phobias, in which clients are exposed to three different feared stimuli presented consecutively over a 3-hour session (e.g., three different dogs in the case of dog phobia) (e.g., Waters et al., 2014; see Ost & Ollendick, 2017 for a review). Future studies should examine whether compound versus consecutive presentations of stimuli during exposure therapy

further enhance physiological arousal yet reduce reactivity to a broader range of generalization stimuli beyond treatment. Furthermore, given that extinction retention effects appear to be strongest when the original CS+ and categorically similar stimuli are included during extinction, yet the original feared stimulus can rarely, if ever, be used during exposure therapy, it may be valuable for future studies to compare imaginal exposure to the CS+ plus actual exposure to the additional stimuli relative to actual exposure to both the CS+ and additional stimuli during extinction.

In summary, this study found that multiple stimuli including the original CSs and novel GSs enhanced physiological arousal during and negative CS evaluations after extinction, yet reduced physiological arousal to perceptually similar novel stimuli and the CS+ after extinction. It is proposed that elevated physiological arousal may be due to enhanced stimulus processing and engagement during extinction, which in turn, enhances the salience of extinction learning, and the recall of extinction memories upon exposure to novel stimuli and re-exposure to the original CS+. Future research should examine whether attention towards and elaborative processing of an even wider range of stimuli underlies enhanced physiological arousal during extinction and reduces physiological arousal to stimuli further along the continuum of perceptual similarity. It would also be informative to assess CS evaluations both trial-by-trial and between phases in future research.

Footnotes

1 Eight trials were initially programmed (four trials of each GS). However, the last two trials were not presented due to a programming error and thus, six trials were presented in total (three trials of each GS).

2 All analyses were initially performed with acquisition CS- responses as a covariate but as no significant effects were observed, the models without the covariate are reported.

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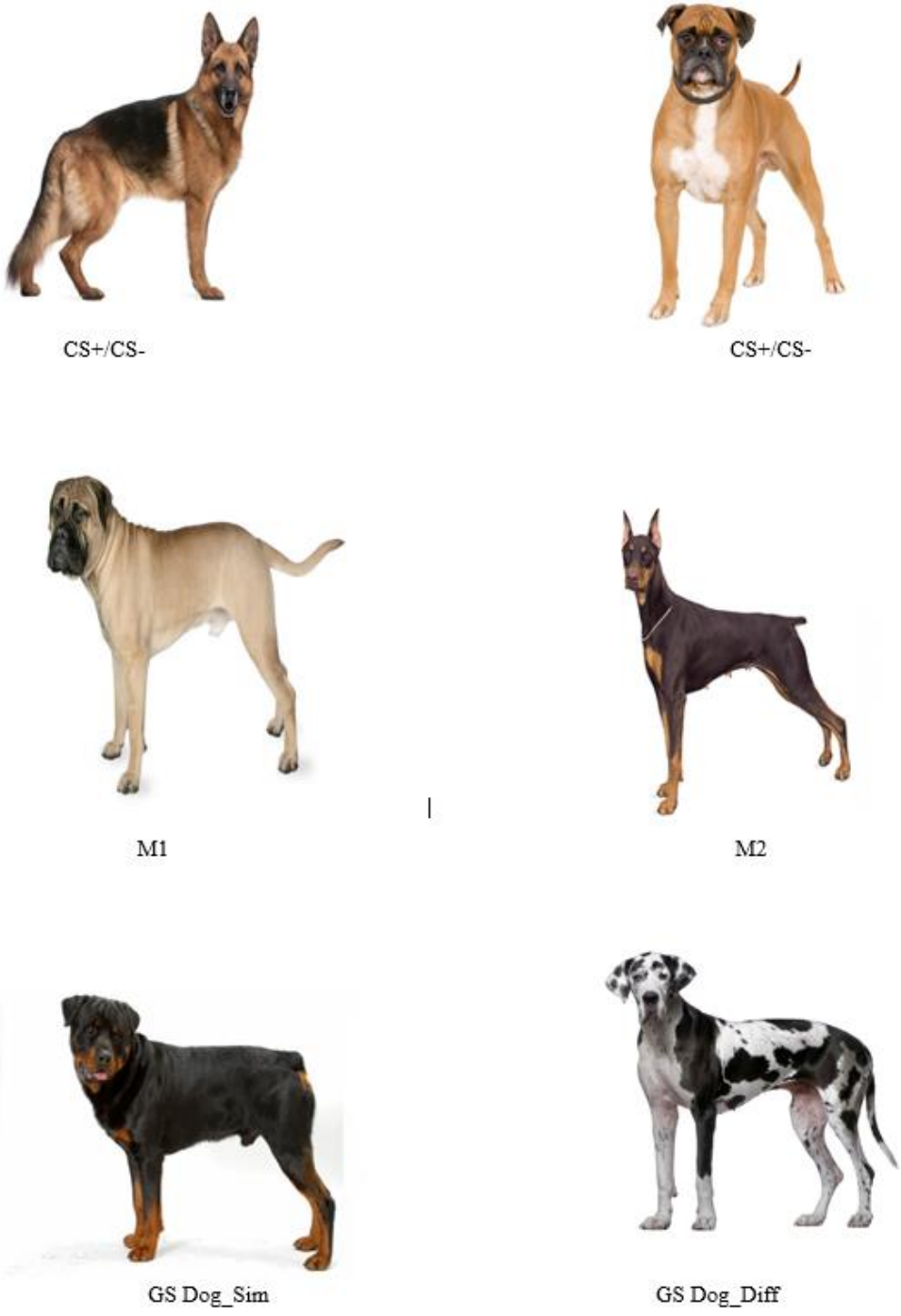

Figure 1: Dog stimuli used in the experiment; CS+/CS- dogs; M1 (Multiple Dog 1), M2 (Multiple Dog 2); GS Dog_Sim (Generalization Dog_Similar), GS Dog_Diff (Generalization Dog_Different).

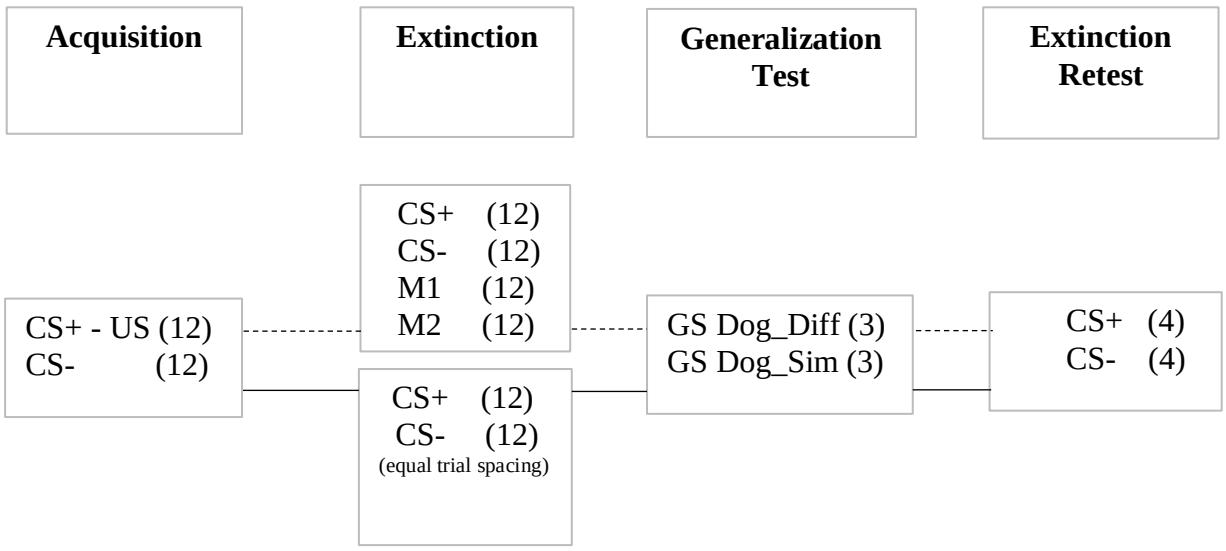

Figure 2: Experimental design

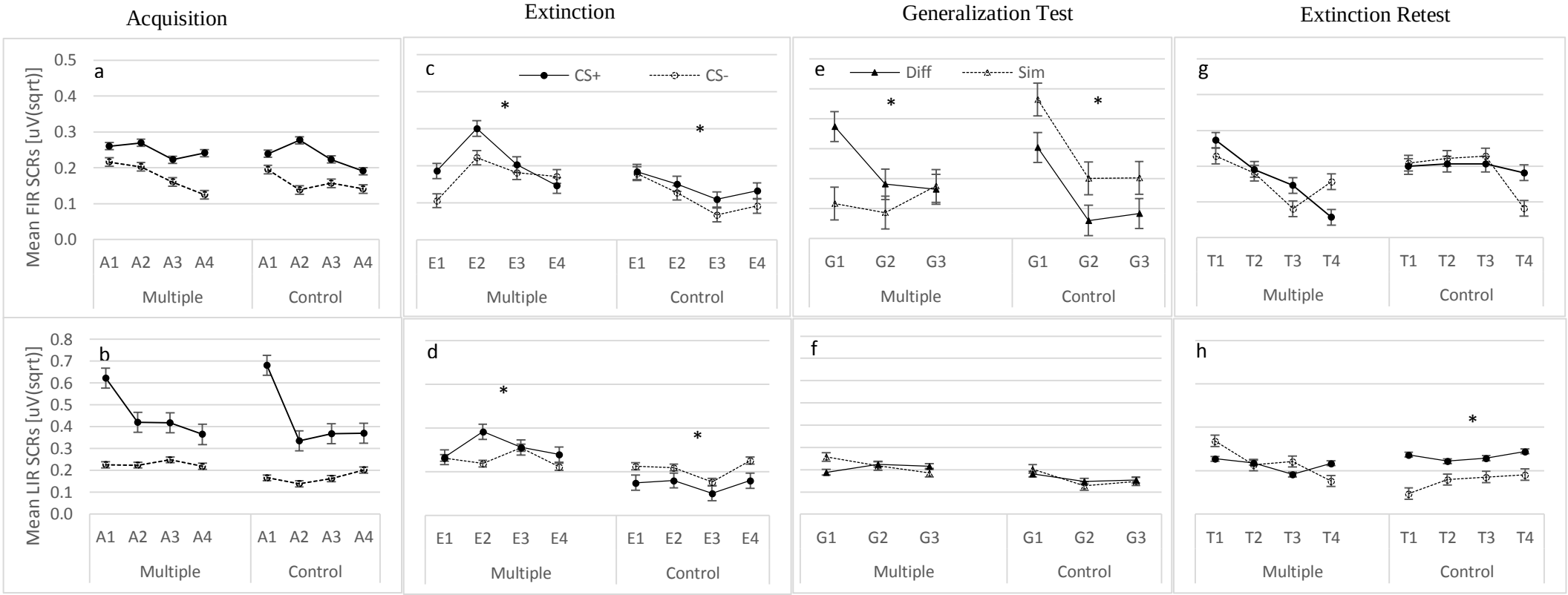

Figure 3. Mean first interval (upper panels) and last interval (lower panels) skin conductance magnitudes (+SE) to the CS+ and the CS- during acquisition, extinction, generalization test, and extinction retest as a function of group. Asterisks indicate significant differences between groups.

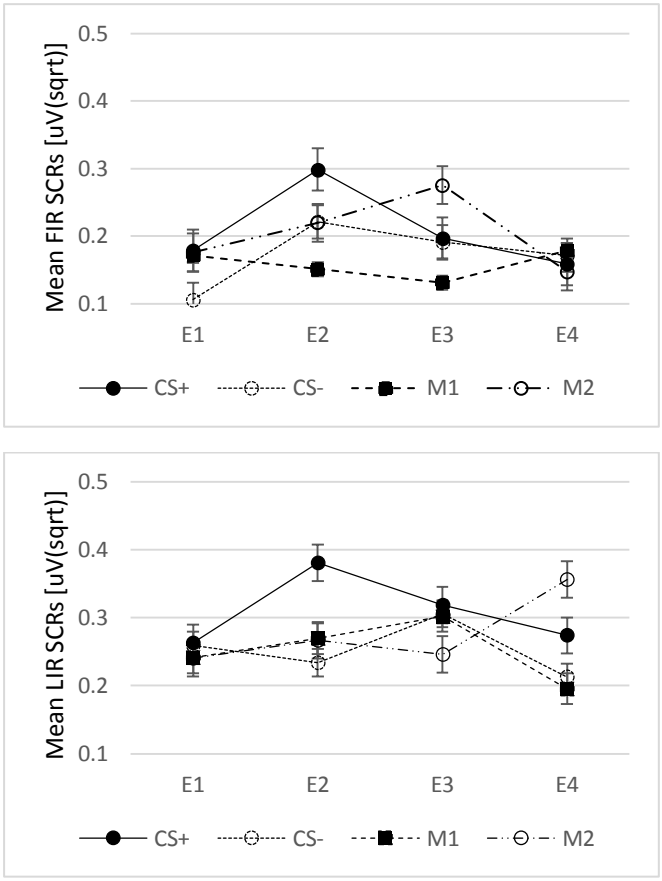

Figure 4. Mean first interval (upper panels) and last interval (lower panels) skin conductance magnitudes (+SE) of the Multiple Group in response to the CSs (CS+, CS-) and GSs (M1, M2) during extinction.

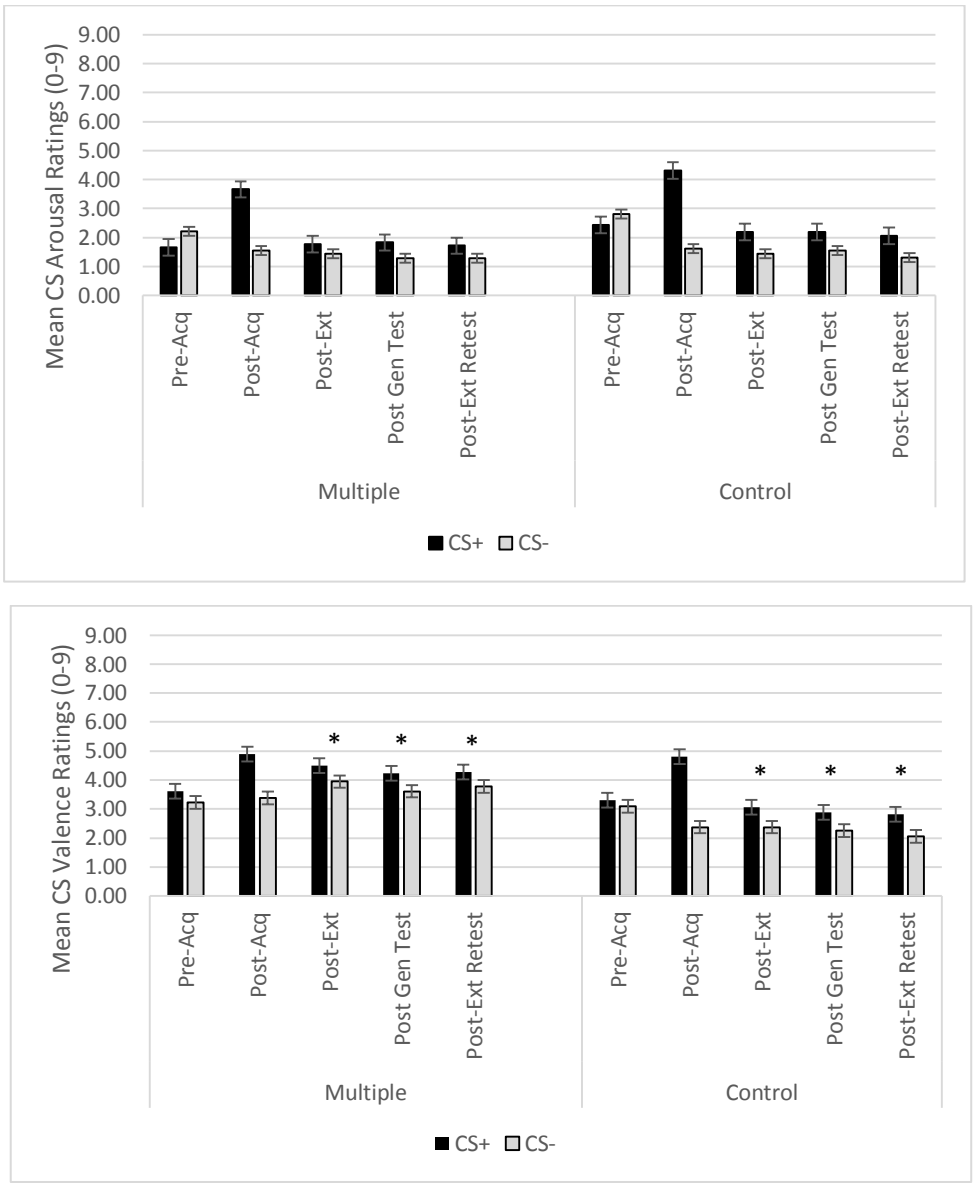

Figure 5. Mean (+SE) CS arousal (upper panel) and valence (lower panel) ratings of the CS+ and CS- before and after acquisition, after extinction, generalization test and extinction retest phases. Asterisks indicate significant differences between groups.

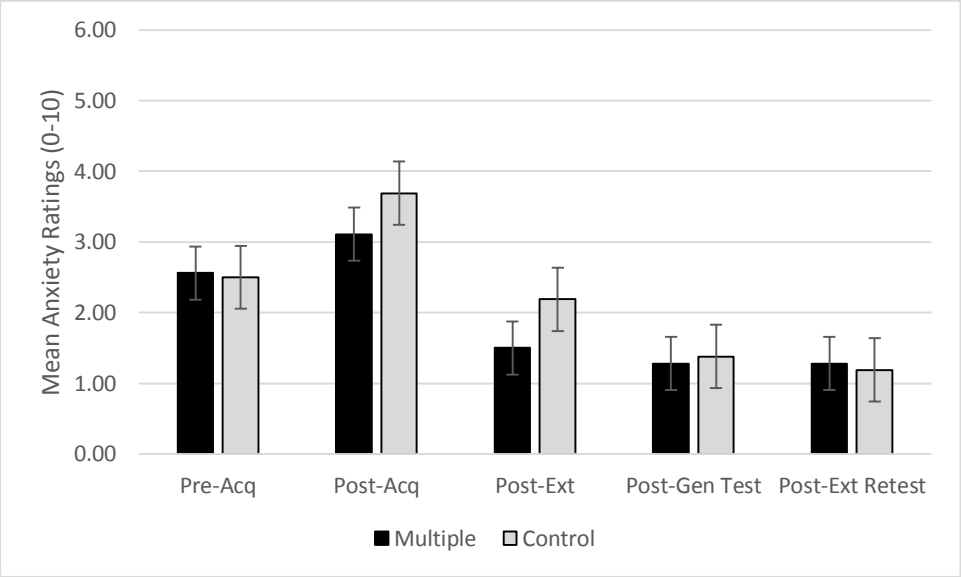

Figure 6. Mean subjective anxiety ratings before and after acquisition, after extinction, generalization test and extinction retest phases as a function of group.