

The role of threat level and intolerance of uncertainty in extinction

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The role of threat level and intolerance of uncertainty in extinction

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Abstract

Recent evidence suggests that individual differences in intolerance of uncertainty (IUS) are associated with disrupted threat extinction. However, it is unknown what maintains the learned threat association in high IUS individuals: is it the experienced uncertainty during extinction or the combination of experienced uncertainty with potential threat during extinction? Here we addressed this question by running two independent experiments with uncertain auditory stimuli that varied in threat level (Experiment 1, aversive human scream ($n = 30$); Experiment 2, neutral tone ($n = 47$) and mildly aversive tone ($n = 49$)). During the experiments, we recorded skin conductance responses and subjective ratings to the learned cues during acquisition and extinction. In experiment 1, high IUS was associated with heightened skin conductance responding to the learned threat vs. safe cue during extinction. In experiment 2, high IUS was associated only with larger skin conductance responding to the learned cues with more threatening properties during extinction i.e. mildly aversive tone. These findings suggest that uncertainty in combination with threat, even when mild, disrupts extinction in high IUS individuals. Such findings help us understand the link between IUS and threat extinction, and its relevance to anxiety disorder pathology.

Keywords: Acquisition, Extinction, Threat, Intolerance of Uncertainty, Skin Conductance

Introduction

Adjusting behaviour based on predictive cues that signal threat and safety is adaptive (LeDoux & Daw, 2018). An organism can learn to associate cues (conditioned stimulus, e.g. a visual stimulus such as a shape) with threatening (unconditioned stimulus, e.g. shock, loud tone) or safe outcomes. Repeated presentations of a cue with a threatening outcome results in defensive responding to the cue alone. This learned association can also be extinguished by repeatedly presenting the conditioned cue without the aversive outcome, resulting in a reduction in defensive responding. Partial reinforcement of aversive stimuli (e.g. shock, noise), particularly at 50% reinforcement rate, has been shown to maintain the conditioned response during extinction (Leonard, 1975; Livneh & Paz, 2012). After partial reinforcement, it is thought that the conditioned response is maintained during extinction due to the uncertainty of receiving a threatening outcome (Bouton, 2002).

Overestimating the predictability of threat over safety is a common feature of anxiety and stress disorders (Duits et al., 2015; Milad & Quirk, 2012). A large body of research has shown that individuals who have anxious traits or who are clinically anxious show reduced extinction of threat, indexed by larger physiological responses to cues that no longer predict an aversive outcome (Etkin & Wager, 2007; Lonsdorf & Merz, 2017). Emerging research from our lab and others suggest that individual differences in intolerance of uncertainty (IUS), the tendency to find uncertainty aversive, may play a specific role in maintaining threat bias during extinction (Dunsmoor, Campese, Ceceli, LeDoux, & Phelps, 2015; Lucas, Luck, & Lipp, 2018; Morriss, Christakou, & van Reekum, 2015, 2016; Morriss, Macdonald, & van Reekum, 2016). For example, after 100% reinforcement, high IUS, relative to low IUS individuals have been found to show generalized skin conductance response

(SCR) across threat and safety cues during early extinction, and to show continued SCR to threat versus safety cues during late extinction (Morriss, Christakou, & van Reekum, 2015, 2016). Moreover, after 50% reinforcement, high IUS has been found to be associated with generalized SCR to parametrically graded stimuli during extinction (e.g. stimuli that vary in similarity to the learned threat cue) (Morriss, Macdonald, & van Reekum, 2016). Individual differences in IUS are typically associated with responding during the extinction phase and not during the acquisition phase (but see Chin et al., 2016; Morriss, Macdonald, & van Reekum, 2016).

During extinction there is a period of uncertainty regarding the change of outcome i.e. threat to safe, and this may induce greater anxiety in high IUS individuals. However, it is unknown whether: (1) high IUS individuals would exhibit disrupted extinction in the absence of threat, as uncertainty (or the omission of information) is aversive enough in itself (Carleton, 2016b), or (2) high IUS individuals would only exhibit disrupted extinction when there is some type of direct threat, even when mild. This question can be examined by varying the level of threat during extinction i.e. manipulating the aversiveness of the US. Given the important role of uncertainty in anxiety (Carleton, 2016a, 2016b; Grupe & Nitschke, 2013) and that current exposure therapies are based on associative learning principles (Craske, Treanor, Conway, Zbozinek, & Vervliet, 2014), examining the parameters by which extinction leads to uncertainty-induced anxiety in high IUS individuals may provide crucial information relevant to anxiety disorder pathology and treatment.

We conducted two experiments using threat and safety cues during acquisition and extinction. For each experiment, we varied the properties of the unconditioned stimulus to assess the relationship between individual differences in

self-reported IUS and the level of threat during extinction. In the first experiment, we aimed to replicate previous IUS and extinction findings using an aversive human scream as the unconditioned stimulus with a 50% reinforcement schedule (Morriss, Christakou, & van Reekum, 2015, 2016; Morriss, Macdonald, & van Reekum, 2016). In the second experiment, we aimed to examine the extent to which IUS would predict reduced extinction when using different unconditioned stimuli that varied in aversiveness i.e. mildly aversive to neutral tones. In experiment 2, we tested two independent samples of participants, with each being presented one of the tones. During both experiments, we measured SCR and expectancy ratings whilst participants performed the acquisition and extinction phases. We used sounds as unconditioned stimuli and visual shape stimuli as conditioned stimuli, similar to previous conditioning research (Neumann, Waters, & Westbury, 2008; Phelps, Delgado, Nearing, & LeDoux, 2004). We used a 50% reinforcement rate during acquisition to maintain conditioning (Leonard, 1975; Livneh & Paz, 2012) and induce greater uncertainty during extinction (Li, Ishii & Naoki, 2016), similar to our previous work (Morriss, Macdonald, & van Reekum, 2016).

In general for experiments 1 and 2, we hypothesised that there would be greater SCR and expectancy ratings to the learned uncertain (threat, mild threat, neutral, also known as the CS+) versus certain (safe, also known as the CS-) cues during acquisition. In addition, for experiment 1, we hypothesised that high IUS would be associated with (1) greater SCR to both the CS+ and CS- cues during early extinction (first 8 CS+/CS- trials), and (2) greater SCR to the CS+ versus CS- during late extinction (last CS+/CS- 8 trials) (Morriss, Christakou, & van Reekum, 2015, 2016), suggesting compromised updating of the CS+ to safe in individuals reporting high IUS. For experiment 2, we had two exploratory hypotheses for IUS and

updating of learned associations during extinction: (1) If uncertainty is aversive enough in itself, we expected high IUS, relative to low IUS, to predict greater SCR to the CS+ versus the CS-, regardless of aversiveness of the unconditioned stimulus. (2) If some level of threat is required, we expected high IUS, relative to low IUS to only predict greater SCR to the CS+ with mild threat versus the CS+ signalling a more neutral outcome (based on the definition of IUS by Carleton, 2016b). For both acquisition and extinction, we tested the specificity of IUS effects by controlling for individual variation reported on the commonly used Spielberger State-Trait Anxiety Inventory, Trait Version (STAI) (Spielberger, Gorsuch, Lushene, Vagg, & Jacobs, 1983). We did not have specific predictions for individual differences in STAI or IUS predicting expectancy ratings, as previous experiments in our lab have not found consistent results for expectancy ratings (Morriss, Christakou, & van Reekum, 2016; Morriss, MacDonald, & van Reekum, 2016).

Experiment 1: Method

Participants

Thirty volunteers (M age = 23.53, SD age = 4.96; 16 females and 14 males) took part in the study¹. All participants had normal or corrected to normal vision.

Participants provided written informed consent and received £5 for their participation.

Advertisements and word of mouth were used to recruit participants from the

University of Reading and local area. The procedure was approved by the University of Reading Research Ethics Committee.

¹ The sample sizes for experiments 1 and 2 were not based on a formal power calculation. However, our sample sizes were matched with comparable experiments using psychophysiological measures to examine conditioning and individual differences in anxiety (e.g. Chin, Nelson, Jackson, & Hajcak, 2016; Morriss, Christakou, & van Reekum, 2016).

Procedure

Participants completed questionnaires online before the study. Participants were invited to participate in the first available experimental. The delay was no longer than 1 week. On the day of the experiment participants arrived at the laboratory and were informed on the experimental procedures. Firstly, participants were seated in the testing booth and asked to complete a consent form as an agreement to take part in the study. Secondly, physiological sensors were attached to the participants' non-dominant hand. The conditioning task (see "Conditioning task" below for details) was presented on a computer, whilst SCR, interbeat interval and behavioural ratings were recorded. Participants were instructed to: (1) maintain attention to the task by looking at the coloured squares and listening to the sounds, which may be unpleasant, (2) respond to the expectancy rating scales that followed each block of trials, using number keys on the keyboard with their dominant hand and (3) to stay as still as possible. The experiment took approximately 30 minutes in total.

Conditioning task

The conditioning task was designed using E-Prime 2.0 software (Psychology Software Tools Ltd, Pittsburgh, PA). Visual stimuli were presented at a 60 Hz refresh rate with a 800 x 600 pixel resolution. Participants sat approximately 60 cm from the screen. Visual stimuli were blue and yellow squares with 183 x 183 pixel dimensions that resulted in a visual angle of 5.78° x 9.73°. The aversive sound stimulus was presented through headphones. The sound consisted of a fear inducing female scream (for sound parameters, see Morriss, Christakou & van Reekum, 2015). The volume of the sound was standardized across participants by using fixed volume

settings on the presentation computer and was verified by an audiometer prior to each session.

The task comprised of two learning phases: acquisition and extinction (see Figure 1). Both acquisition and extinction consisted of two blocks. In acquisition, one of the coloured squares (blue or yellow) was paired with the aversive 90 dB sound 50% of the time (CS+), whilst the other square (yellow or blue) was presented alone (CS-). The 50% pairing rate was designed to maximize uncertainty of the CS+ / US contingency. During extinction, both the blue and yellow squares were presented in the absence of the US.

The acquisition phase consisted of 24 trials (6 CS+ paired, 6 CS+ unpaired, 12 CS-) and the extinction phase 32 trials (16 CS+ unpaired, 16 CS-). Early extinction was defined at the first 8 CS+/CS- trials and late extinction was defined as the last 8 CS+/CS- trials. Experimental trials were pseudo-randomized such that the first trial of acquisition was always paired and then after all trial types were randomly presented within blocks (Block 1: 2 CS+ paired, 3 CS+ unpaired, 6 CS-; Block 2: 3 CS+ paired, 3 CS+; 6 CS-; Blocks 3 and 4: 8 CS+, 8 CS-). Conditioning contingencies were counterbalanced, with half of participants receiving the blue square paired with the US and the other half of participants receiving the yellow square paired with the US. The coloured squares were presented for a total of 4000 ms. The aversive sound lasted for 1000 ms, which coterminated with the reinforced CS+s. Subsequently, a blank screen was presented for 6000 – 8800 ms, similar to previous work (Morriss, Chapman, Tomlinson & van Reekum, 2018). Given that we compare levels of responding across conditions of the experiment (Breska, Maoz, & Ben-Shakhar, 2011), we used shorter intertrial intervals to reduce the overall duration of the experiment and to avoid fatigue or boredom in the participant, which

is in line with recent recommendations and considerations for fear conditioning designs involving SCR (Lonsdorf et al., 2017).

At the end of each block (4 blocks in total, 2 in acquisition and 2 in extinction), participants were asked to rate how much they expected the blue square and yellow square to be followed by the sound stimulus, where the scale ranged from 1 (“Don’t Expect”) to 9 (“Do Expect”). Ratings were obtained at the end of each block versus each trial to reduce the length of the experiment, reduced movement artefacts in the skin conductance signal and to avoid explicit focus on expectancy.

Two other 9-point Likert scales were presented at the end of the experiment. Participants were asked to rate the valence and arousal of the sound stimulus. The scales ranged from 1 (Valence: negative; Arousal: calm) to 9 (Valence: positive; Arousal: excited).

Questionnaires

To assess anxious disposition, we administered the Spielberger State-Trait Anxiety Inventory – Trait version (STAI) and Intolerance of Uncertainty Scale (IUS) (Freeston, Rhéaume, Letarte, Dugas, & Ladouceur, 1994). The IUS measure consists of 27 items with a five-point Likert scale, example items include “Uncertainty makes me uneasy, anxious, or stressed” and “I must get away from all uncertain situations”. The STAI measure consists of 20 items with a four-point Likert scale. Similar distributions and internal reliability of scores were found for the anxiety measures, STAI ($M = 41.30$; $SD = 9.84$; range = 26-56; $\alpha = .91$), IUS ($M = 67.50$; $SD = 17.18$; range = 33-94; $\alpha = .93$).

Behavioural data scoring

Rating data were reduced for each participant by calculating their average responses for each experimental condition (Acquisition CS+; Acquisition CS-; Extinction CS+ Early; Extinction CS- Early; Extinction CS+ Late; Extinction CS- Late) using the E-Data Aid tool in E-Prime (Psychology Software Tools Ltd, Pittsburgh, PA).

Physiological acquisition and scoring

Physiological recordings were obtained using AD Instruments (AD Instruments Ltd, Chalgrove, Oxfordshire) hardware and software. Electrodermal activity was measured with dry MLT116F silver/silver chloride bipolar finger electrodes that were attached to the distal phalanges of the index and middle fingers of the non-dominant hand. A low constant-voltage AC excitation of 22 mV_{rms} at 75 Hz was passed through the electrodes, which were connected to a ML116 GSR Amp, and converted to DC before being digitized and stored. Interbeat Interval (IBI) was measured using a MLT1010 Electric Pulse Transducer, which was connected to the participant's distal phalange of the ring finger. An ML138 Bio Amp connected to an ML870 PowerLab Unit Model 8/30 amplified the SCR and IBI signals, which were digitized through a 16-bit A/D converter at 1000 Hz. IBI signal was used only to identify movement artefacts and was not analysed. The electrodermal signal was converted from volts to microSiemens using AD Instruments software (AD Instruments Ltd, Chalgrove, Oxfordshire).

CS+ unpaired and CS- trials were included in the analysis, but CS+ paired trials were discarded to avoid sound confounds. SCR's were scored when there was an increase of skin conductance level exceeding 0.03 microSiemens. The amplitude of each response was scored as the difference between the onset and the maximum deflection prior to the signal flattening out or decreasing. SCR onsets and respective

peaks were counted if the SCR onset was within 0.5-3.5 seconds following CS onset. Trials with no discernible SCRs were scored as zero (Morriss, Chapman, Tomlinson, & van Reekum, 2018). SCR's were square root transformed to reduce skew at the trial level (Dawson, Schell, & Filion, 2000) and were z-scored to control for interindividual differences in skin conductance responsiveness (Ben-Shakhar, 1985). SCR magnitudes were calculated by averaging the transformed values for each condition, creating the following conditions: Acquisition CS+; Acquisition CS-; Extinction CS+ Early; Extinction CS- Early; Extinction CS+ Late; Extinction CS- Late. In acquisition, 45% of trials (CS+ unpaired, CS-) were scored as zero and in extinction 54% of trials (CS+ unpaired, CS-) were scored as zero.

SCR magnitude inclusion

In the sample, we had one non-responder, defined as having less than 10% of SCR responses to unpaired trials across acquisition and extinction. We report below the SCR magnitude results without the non-responder included.²

Ratings and SCR magnitude analysis

The analysis was conducted using the mixed procedure in SPSS 21.0 (SPSS, Inc; Chicago, Illinois). We conducted separate multilevel models on ratings and SCR magnitude for each phase (Acquisition, Extinction). For ratings and SCR magnitude during the acquisition phase we entered Stimulus (CS+, CS-) at level 1 and individual subjects at level 2. For ratings and SCR magnitude during the extinction phase we entered Stimulus (CS+, CS-) and Time (Early, Late) at level 1 and

² For experiment 1 the SCR magnitude results do not change if: (1) the non-responder is included [Stimulus x IUS, $F(1, 112.367) = 8.043, p = .005$], and (2) IUS is entered alone into the MLM [Stimulus x IUS, $F(1, 109.538) = 8.782, p = .004$].

individual subjects at level 2. We included the following individual difference predictor variables into the multilevel models: IUS and STAI. In all models, we used a diagonal covariance matrix for level 1. Random effects included a random intercept for each individual subject, where a variance components covariance structure was used. Fixed effects included Stimulus, Phase and Time. We used a maximum likelihood estimator for the multilevel models. We used the least significance difference procedure for pairwise comparisons.

In the model where there are two predictor variables (IUS, STAI), a significant interaction with one variable but not the other suggests specificity. Based on our prior work, we expected such specificity for IUS, but we explored interactions with STAI, given extant findings with STAI in the conditioning literature (e.g. Lonsdorf & Merz, 2017). Where a significant interaction was observed with IUS (or STAI), we performed follow-up pairwise comparisons on the estimated marginal means of the relevant conditions estimated at specific IUS values of + or -1 SD of mean IUS, adjusted for STAI (or IUS). These data are estimated from the multilevel model of the entire sample, not unlike performing a simple slopes analysis in a multiple regression analysis. Similar analyses have been published elsewhere (Morriss, Macdonald, & van Reekum, 2016; Morriss, McSorley, & van Reekum, 2017).

Experiment 1: Results

For descriptive statistics see Table 1.

Ratings

Participants rated the human scream sound stimulus as aversive ($M = 2.43$ $SD = 1.41$, where 1 = negative and 9 = positive) and arousing ($M = 6.50$, $SD = 1.78$, where 1 = calm and 9 = excited).

Participants had higher expectancy ratings of the sound with the CS+ versus CS- during acquisition [Stimulus, $F(1, 30) = 16.075$, $p < .001$] and extinction [Stimulus, $F(1, 81.159) = 65.290$, $p < .001$; see Table 1]. In the early part of extinction, participants displayed higher expectancy ratings of the sound with the CS+ versus CS-, $p = .001$. However, during late extinction, the expectancy rating of the sound with the CS+ dropped and was similar to the CS-, $p = 1$ [Time, $F(1, 81.159) = 154.667$, $p < .001$; Stimulus x Time, $F(1, 81.159) = 65.290$, $p < .001$].

During acquisition, individuals scoring lower in STAI tended to have greater discrimination between expectancy of the sound with the CS+ ($M = 5.05$, $SE = .41$) versus CS- ($M = 2.70$, $SE = .54$), $p < .001$, whilst individuals with higher STAI tended to have poorer discrimination between expectancy of the sound with the CS+ ($M = 3.82$, $SE = .41$) and CS- ($M = 3.96$, $SE = .54$), $p = .781$ [Stimulus x STAI, $F(1, 30) = 4.141$, $p = .026$]. Moreover, during extinction, the same pattern of discrimination was observed, as low STAI showed greater discrimination, $p < .001$ (CS+: $M = 4.32$, $SE = .44$; CS-: $M = 1.58$, $SE = .46$), compared to high STAI. $p = .02$ (CS+: $M = 4.33$, $SE = .44$; CS-: $M = 3.25$, $SE = .46$) [Stimulus x STAI, $F(1, 81.159) = 4.493$, $p = .037$]. A similar pattern was observed for IUS during extinction [Stimulus x IUS, $F(1, 81.159) = 4.146$, $p = .045$]. No other significant main effects or interactions with STAI or IUS were found, max $F = 3.780$.

SCR magnitude

Larger average SCR magnitude was found for the CS+, compared to the CS- during acquisition [Stimulus, $F(1, 29) = 8.701, p = .006$]. Unexpectedly, during acquisition, high STAI was associated with greater SCR magnitude to CS+ ($M = .254, SE = .101$) vs. CS- ($M = -.129, SE = .069$), $p = .003$, whilst low STAI was associated with reduced SCR magnitude difference between CS+ ($M = .184, SE = .101$) vs. CS- ($M = .202, SE = .069$), $p = .878$ [Stimulus x STAI, $F(1, 29) = 4.294, p = .023$]³. Individual differences in IUS were not associated with SCR during this phase.

During extinction, larger SCR magnitude was observed for the CS+ versus CS- [Stimulus, $F(1, 108.786) = 5.167, p = .025$; see Table 1]. Partially in line with our predictions, higher IUS was associated with greater SCR magnitude response to the CS+ versus CS- during extinction, $p < .001$, whilst lower IUS was associated with no significant differential SCR magnitude response between the CS+ and CS-, $p = .218$ [Stimulus x IUS, $F(1, 108.786) = 8.351, p = .005$] (see Figure 2). Time (early vs late) did not affect this relationship, however. No other significant main effects or interactions with IUS or STAI were found, max $F = 2.129$.

Experiment 1: Conclusion

For experiment 1 we observed typical profiles of acquisition and extinction, where larger SCR magnitudes and expectancy ratings were found for the CS+ vs. CS-. High IUS was associated with larger SCR magnitude to the CS+ vs. CS- during extinction. This finding partially replicates our previous research (Morris, Christakou, & Van Reekum, 2015, 2016), as we did not observe time-based effects of IUS and threat extinction. Nevertheless, these findings further support the notion

³ The effect of Stimulus x STAI during acquisition was only observed when IUS was included in the model.

that threat extinction is inherently uncertain and induces greater anxiety-related arousal in individuals with high IUS. Building upon these findings, in the next experiment we assess whether: (1) high IUS individuals exhibit disrupted extinction in the absence of direct threat (Carleton, 2016b), or (2) high IUS individuals only exhibit disrupted extinction when there is some type of direct threat, even when mild. We address this question by varying the level of threat during extinction i.e. manipulating the aversiveness of the US. Furthermore, we assess the specificity of IUS-related results against STAI.

Surprisingly, we observed greater discrimination of expectancy ratings of the sound with the CS+ vs. CS- during acquisition and extinction for individuals lower in STAI. In addition, during acquisition individuals lower in STAI showed reduced discrimination in SCR magnitude for the CS+ vs. CS-. However, the latter effect was not observed for SCR magnitude when STAI was entered alone in the model. In the next experiment, we assess whether similar results are obtained for STAI when the US is less threatening.

Experiment 2: Method

All aspects of the method are identical to experiment 1, except the following below.

Participants

Ninety six volunteers (M age = 19.59, SD age = 1.93; 81 females and 15 males) took part in the study. The neutral tone group, $N=47$ (M age = 19.28, SD age = 1.16; 38 females and 9 males), and aversive tone group, $N = 49$ (M age = 19.89, SD age = 2.43; 43 females and 6 males) underwent similar conditioning procedures, but received different US stimulation (see “Conditioning task” below for details). All

participants had normal or corrected to normal vision. Participants provided written informed consent and received 0.5 credits for their participation. The procedure was approved by the University of Reading Research Ethics Committee.

Procedure

On the day of the experiment participants arrived at the laboratory and were informed on the experimental procedures. Firstly, participants were seated in the testing booth and asked to complete a consent form as an agreement to take part in the study and a set of questionnaires on the computer (see below). To ensure a comparable distribution of IUS scores, participants with low (below average < 65) and high IUS (above average > 65) were evenly distributed to the neutral tone and aversive tone groups (similar to previous work, Morriss & van Reekum, 2019). Next, physiological sensors were attached to the participants' non-dominant hand. The conditioning task (see "Conditioning task" below for details) was presented on a computer, whilst SCR, interbeat interval (to help in artefact detection) and behavioural ratings were recorded. Participants were instructed to: (1) maintain attention to the task by looking at the coloured squares and listening to the sounds, which may be unpleasant, (2) respond to the expectancy rating scales that followed each block of trials, using number keys on the keyboard with their dominant hand and (3) to stay as still as possible. The experiment took approximately 30 minutes in total.

Conditioning task

The conditioning task procedure in experiment 2 was similar to experiment 1. Visual stimuli were blue and yellow squares presented on a computer screen and served as

CSs (see “Experiment 1: Method”, “Conditioning task” section for more details). The aversive sound stimulus was presented through headphones and served as US. Each experimental group received a different auditory stimulus. The Aversive Tone Group was exposed to a high pitched tone (1600 Hz, 1000 ms, 90 db). The Neutral Tone Group was exposed to a low pitched tone (360 Hz, 1000 ms, 80 db). We used Audacity 2.0.3 software (<http://audacity.sourceforge.net/>) to generate the tones. The volume of the sound was standardized across participants by using fixed volume settings on the presentation computer and was verified by an audiometer prior to each session.

Questionnaires

Similar distributions and internal reliability of scores were found for the anxiety measures. For the neutral tone group: STAI ($M = 45.40$; $SD = 9.78$; range = 29-66; $\alpha = .91$), IUS ($M = 68.21$; $SD = 15.04$; range = 42-101; $\alpha = .90$). For the aversive tone group: STAI ($M = 42.55$; $SD = 10.89$; range = 26-70; $\alpha = .92$), IUS ($M = 67.94$; $SD = 15.59$; range = 42-110; $\alpha = .91$). The groups did not significantly differ on STAI [$t(92) = .1.335$, $p = .185$] or IUS scores [$t(94) = .088$, $p = .930$].

Physiological scoring

For the neutral tone group, in acquisition, 72% of trials were scored as zero and in extinction 73% of trials were scored as zero. For the aversive tone group, in acquisition, 71% of trials were scored as zero and in extinction 72% of trials were scored as zero.

SCR magnitude inclusion

Based on the criterion specified in experiment 1, the neutral tone group had seven non-responders, and the aversive tone group had ten non-responders. This left forty participants in the neutral tone group and thirty-nine participants in the aversive tone group with usable SCR data. We report below the SCR magnitude results excluding the non-responders.⁴

Ratings and SCR magnitude analysis

The same statistical procedures from Experiment 1 were used in Experiment 2. IUS and STAI were entered as continuous predictor variables. We added an additional factor of Group (Neutral tone, Aversive tone).

Experiment 2: Results

For descriptive statistics see Table 2.

Ratings

In the neutral tone group, the sound was rated as slightly aversive ($M = 4.08$, $SD = 1.28$, where 1 = negative and 9 = positive) and neutral in arousal ($M = 5.02$, $SD = 1.76$, where 1 = calm and 9 = excited). In the aversive tone group, the sound was rated as moderately aversive ($M = 3.42$, $SD = 1.45$, where 1 = negative and 9 = positive) and arousing ($M = 5.97$, $SD = 1.78$, where 1 = calm and 9 = excited). The aversive tone was rated significantly more aversive [$t = -2.636$, $p = .010$] and arousing [$t = 2.339$, $p = .021$] than the neutral tone.

⁴ For experiment 2, the SCR magnitude results do not change if: (1) the non-responders are included [Stimulus x Group x IUS, $F(1, 356.280) = 4.502$, $p = .035$] and (2) IUS is entered alone into the MLM [Stimulus x IUS, $F(1, 298.580) = 10.425$, $p = .001$].

Participants had higher expectancy ratings of the tones with the CS+ versus CS- during acquisition [Stimulus, $F(1, 175.914) = 339.935, p < .001$; see Table 2]. No other significant effects of Group or interactions with Group, IUS or STAI were observed during acquisition, Max $F = 1.021$. Similar patterns of ratings during extinction were observed for the neutral and aversive tone groups [Stimulus, $F(1, 240.054) = 94.134, p < .001$; Time, $F(1, 240.054) = 40.569, p < .001$; Stimulus x Time, $F(1, 240.054) = 13.329, p < .001$]. Participants displayed higher expectancy ratings of the tones with the CS+ versus CS- during early extinction, compared to late extinction, p 's $< .001$.

Surprisingly, during extinction, an effect of STAI was found [Stimulus x STAI, $F(1, 240.054) = 3.961, p = .048$], where low STAI was associated with greater discrimination of expectancy of the tones with the CS+ ($M = 2.98, SE = .26$) vs. CS- ($M = 1.54, SE = .24$), $p < .001$, compared to high STAI, $p < .001$ (CS+: $M = 2.42, SE = .26$; CS-: $M = 1.62, SE = .24$). No other significant main effects or interactions were found during extinction, max $F = 2.907$.

SCR magnitude

Greater SCR magnitude was found for the CS+, compared to the CS- during acquisition [Stimulus, $F(1, 71) = 4.719, p = .033$; see Table 2]. No other significant main effects of Group or interactions with IUS or STAI emerged during acquisition, max $F = 1.105$.

Larger SCR magnitude was found for the CS+, compared to the CS- during extinction [Stimulus, $F(1, 287.063) = 5.342, p = .022$]. Notably, tone group and individual differences in IUS predicted SCR magnitude during extinction [Stimulus x Group x IUS, $F(1, 287.063) = 6.410, p = .012$]. In the aversive tone group, higher IUS

was associated with greater SCR magnitude response during extinction to the CS+ versus CS-, $p = .004$, whilst lower IUS was associated with no significant differential SCR magnitude response during extinction between the CS+ and CS-, $p = .415$ (see Figure 3). Interestingly, in the neutral tone group, higher IUS was associated with no significant differential SCR magnitude response during extinction to the CS+ versus CS-, $p = .815$, whilst lower IUS was associated with a significant differential SCR magnitude response during extinction between the CS+ and CS-, $p = .036$.

No other significant main effects of Group or interactions with IUS or STAI emerged, max $F = 3.655$.

Experiment 2: Conclusion

For experiment 2 we observed typical profiles of acquisition and extinction, where larger SCR magnitudes and expectancy ratings were found for the CS+ vs. CS-, despite the different threat levels of the US. High IUS was only associated with larger SCR magnitude to the CS+ versus CS- during extinction for the mildly aversive tone group. Conversely, low IUS individuals in the neutral tone group displayed larger SCR magnitude to the CS+ versus CS- during extinction. Overall, these findings suggest that uncertainty in combination with threat, even when mild, continues to disrupt extinction in high IUS individuals.

Similar to experiment 1, low STAI was associated with greater discrimination of expectancy of the tones with the CS+ vs. CS- during extinction. This effect suggests that STAI may be more related to expectancy biases in general, regardless of the level of threat.

General Discussion

In the current study, we show that differences in self-reported IUS are related to extinction depending on the level of uncertain threat present. These results partially replicate and extend prior findings from our lab of bodily and neural responding associated with IUS and threat extinction (Morriss, Christakou, & van Reekum, 2015, 2016; Morriss, Macdonald, & van Reekum, 2016). Importantly, these findings provide another piece of the puzzle in recognising the relevance of IUS-related mechanisms in disrupting threat extinction, which will likely have implications for anxiety disorder pathology and exposure-based treatment.

For both experiments we observed typical patterns of acquisition and extinction, where larger SCR magnitudes and expectancy ratings were found for the CS+ vs. CS-. In the first experiment, we aimed to examine the effect of an aversive uncertain US (i.e. human scream) on threat extinction and individual differences in IUS. The aversive US was presented with a 50% reinforcement schedule during acquisition. High IUS was associated with larger SCR magnitude to CS+ versus CS- cues during extinction. This finding is line with previous research examining IUS and threat extinction (Dunsmoor, Campese, Ceceli, LeDoux, & Phelps, 2015; Lucas, Luck, & Lipp, 2018; Morriss, Christakou, & van Reekum, 2015, 2016; Morriss, Macdonald, & van Reekum, 2016).

In the second experiment, we kept the same partial reinforcement procedure but changed the aversiveness of the US: One group of participants received a neutral tone and another group of participants received a mildly aversive tone. On average participants rated the aversive tone as more aversive and arousing than the neutral tone. The majority of participants rated the aversive tone as mildly aversive i.e. 40 out of 49 rated the sound as less than 5 on the valence scale. However, the valence ratings for the neutral tone were variable i.e. 28 out of 47 rated the sound as

less than 5 on the valence scale. Therefore, the neutral tone was aversive to some individuals. Despite these differences, high IUS was only associated with larger SCR magnitude to the learned cues during extinction for the mildly aversive tone group. Interestingly, individuals reporting *low* IUS in the neutral tone group displayed larger SCR magnitude to the CS+ versus CS- during extinction. It is possible that low IUS individuals may have preferred to have the neutral tone over nothing in the extinction phase, as they may have found the neutral tone pleasant or positively stimulating, versus the boredom of having nothing.

The observed IUS-related effects on SCR magnitude during extinction for experiment one and two were specific to IUS, over STAI. Taken together, the results from experiment one and two suggest that uncertain threat, even when it is mild, is an important factor in disrupting extinction in high IUS individuals, as indexed by SCR. From a clinical perspective, these findings are particularly interesting, as associative learning principles underlie exposure-based therapies (Bouton, 1988; Craske et al., 2014). For example, we can speculate that patients undergoing exposure therapy may require a different number of sessions depending on their IUS score and the perceived aversiveness of the conditioned stressor(s).

The findings reported here feed into a broader research context examining the role of IUS in anxiety and stress disorders (Carleton, 2016a; Carleton, 2016b; Grupe & Nitschke, 2013; Tanovic, Gee & Joorman, 2018). The majority of work on IUS has focused on the processing of threat (Carleton, 2016a; Carleton, 2016b; Grupe & Nitschke, 2013; Tanovic, Gee & Joorman, 2018). However, a few studies have also examined IUS in relation to reward and neutral contexts. These studies highlight that high IUS is associated with reduced responding to anticipating and receiving rewards (Gorka, Nelson, Phan & Shankman, 2016; Nelson, Shankman & Proudfit, 2014).

Moreover, high IUS is associated with reduced attentional inhibition during uncertain contexts in the absence of direct threat or reward (Morriss & McSorley, 2019). More empirical work is needed to clarify why uncertainty in the absence of threat is aversive (Shihata et al., 2016). For example, it will be important to identify whether distinct psychological mechanisms are modulated by uncertain threat and reward differently e.g. associative learning of threat vs. reward, attentional inhibition of threat vs. reward. This will provide insights into whether a given mechanism is: (1) equally modulated by uncertainty in the absence and presence of threat or reward, or (2) linearly modulated by uncertainty depending on the level of threat or reward. Examining these aspects of IUS will inform future IUS theory and research.

In the current experiments we did not observe time-based effects of IUS and threat extinction (Morriss, Christakou, & van Reekum, 2015, 2016). The difference between these experimental findings may be due to the reinforcement rate and timing of the CS. In the current experiments, we used a 50% reinforcement rate during the acquisition phase, whilst in our original experiments the rate was 100%. We used a 50% reinforcement rate to: (1) increase uncertainty during acquisition and subsequent extinction (Bouton, 2002), and (2) assess the conditioned response without the confound of the US. In addition, the current experiments used a CS of 4 seconds, whilst in our original experiments the CS was 1.5 seconds. It is advantageous to use a CS with a longer duration as it allows for more SCRs to be captured across all trials. Despite these design differences, IUS-related effects were still observed in extinction.

Interestingly, our IUS-related results differed depending on the type of measurement we used. The IUS-related results in extinction were consistent for SCR magnitude across experiments one and two. The majority of research examining the

effects of IUS on threat acquisition and extinction have found significant relationships between IUS and psychophysiological measures such as startle and SCR (Chin, Nelson, Jackson, & Hajcak, 2016; Morriss, Christakou, & van Reekum, 2015, 2016; Morriss, Macdonald, & van Reekum, 2016; Morriss, McSorley, & van Reekum, 2017; Sjouwerman, Scharfenort, & Lonsdorf, 2017). For the ratings we observed results with STAI, over IUS, in experiment one and two. In experiment one, for both acquisition and extinction, individuals scoring higher on STAI tended to have higher ratings of expectancy for both the CS+ and CS-, whilst individuals lower on STAI showed greater discrimination between expectancy of the CS+ versus CS-. In experiment two, STAI significantly predicted the expectancy ratings during extinction. These results suggest that STAI may be more generally related to subjective ratings, and possibly specifically to ratings of expectancy, regardless of the level of threat during extinction. To our knowledge only a few studies have observed IUS effects on ratings during acquisition and extinction (Morriss, Macdonald, & van Reekum, 2016; Sjouwerman, Scharfenort, & Lonsdorf, 2017). The lack of consistent patterns between psychophysiological and rating measures for IUS may, at least in our studies, also be due to the time between phasic cue events and rating periods in the experiment, where recall of expectancy was required for each block at the moment of rating.

A few issues with the current experiments should be further addressed in future research to assess the robustness and generalizability of the findings reported here. Firstly, the effect of threat level should be tested using multiple stimuli that vary linearly in averseness, ideally within the same experiment. Secondly, other types of threat should be tested e.g. level of shock, fearful/angry faces. Thirdly, the sample contains mainly young female participants and there are known effects of sex and

age on threat extinction (Merz, Kinner & Wolf, 2018; Shechner, Hong, Britton, Pine, & Fox, 2014). Therefore, future studies should look to replicate these findings in more diverse samples. Fourthly, in experiment two there was a higher percentage of SCR's scored as zero and more non-responders, which are likely due to the aversiveness of the US (i.e., the tones were rated as less aversive than the scream), but we cannot rule out effects of random sample variation across our experiments. Lastly, our results differed depending on which version of the IUS scale was entered into the analyses.⁵ We recommend future work to compare the 27-item and 12-item IUS scales (Carleton, Norton, & Asmundson, 2007; Khawaja, & Yu, 2010) as this may reveal which of the scales are more reliable for predicting particular behaviours.

In conclusion, these initial results provide some insight into how threat level and extinction may be related to IUS, which may be relevant for understanding uncertainty-induced anxiety and relevant treatment targets (Carleton, 2016a, 2016b; Grupe & Nitschke, 2013). Further research is needed to assess how individual differences in IUS modulate learned associations that vary in valence and arousal.

⁵ For experiment 1, the SCR magnitude results were the same when the IUS-12 was used instead of the IUS-27, [Stimulus x IUS-12, $F(1, 108.416) = 11.874, p = .001$]. For experiment 2, the SCR magnitude results were no longer significant when the IUS-12 was used instead of the IUS-27, [Stimulus x Group x IUS-12, $F(1, 285.753) = .354, p = .552$].

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Table 1. Experiment 1 summary of means (SD) for each dependent measure as a function of condition (CS+ and CS-), separately for acquisition, early extinction and late extinction.

Measure	Acquisition		Early Extinction		Late Extinction	
	CS+	CS-	CS+	CS-	CS+	CS-
Square root transformed and z-scored SCR magnitude ($\sqrt{\mu\text{S}}$)	0.22 (0.30)	0.03 (0.22)	0.09 (0.44)	-0.16 (0.41)	-0.05 (0.31)	-0.11 (0.43)
Expectancy rating (1-9)	4.43 (1.26)	3.33 (1.62)	6.77 (1.22)	2.93 (1.89)	1.90 (1.65)	1.90 (1.92)

Note: SCR magnitude ($\sqrt{\mu\text{S}}$), square root transformed and z-scored skin conductance magnitude measured in microSiemens.

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Table 2. Experiment 2 summary of means (SD) for each dependent measure as a function of group (Neutral Tone and Aversive Tone) and condition (CS+ and CS-), separately for acquisition, early extinction and late extinction.

Group	Measure	Acquisition		Early Extinction		Late Extinction	
		CS+	CS-	CS+	CS-	CS+	CS-
Neutral Tone	Square root transformed and z-scored SCR magnitude ($\sqrt{\mu\text{s}}$)	0.17 (0.43)	0.01 (0.28)	0.2 (0.38)	-0.11 (0.28)	-0.01 (0.44)	-0.04 (0.39)
	Expectancy rating (1-9)	5.86 (1.49)	1.84 (1.39)	3.28 (2.00)	1.66 (1.59)	2.11 (1.49)	1.49 (1.23)
Aversive Tone	Square root transformed and z-scored SCR magnitude ($\sqrt{\mu\text{s}}$)	0.16 (0.57)	0.07 (0.30)	0.01 (0.29)	-0.02 (0.31)	-0.04 (0.40)	-0.18 (0.30)
	Expectancy rating (1-9)	5.99 (1.55)	1.84 (1.53)	3.20 (1.72)	1.76 (1.85)	2.10 (1.58)	1.35 (1.30)

Note: SCR magnitude ($\sqrt{\mu\text{S}}$), square root transformed and z-scored skin conductance magnitude measured in microSiemens.

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Figure Captions

Fig 1. Image depicting the acquisition (with 50% reinforcement) and extinction phases of experiment 1 and 2. In experiment 1, the unconditioned stimulus was a female scream. In experiment 2, the unconditioned stimulus was either a neutral or mildly aversive tone.

Fig 2. Bar graphs depicting IUS estimated at + or - 1 SD of mean IUS (controlling for STAI) from the multilevel model analysis for SCR magnitude during extinction. In experiment 1, high IUS, relative to low IUS individuals were found to show heightened skin conductance responding to the CS+ versus CS- cue during extinction. Bars represent standard error at + or - 1 SD of mean IUS. Square root transformed and z-scored SCR magnitude (μS), skin conductance magnitude measured in microSiemens.

Fig 3. Bar graphs depicting IUS estimated at + or - 1 SD of mean IUS (controlling for STAI) from the multilevel model analysis for SCR magnitude during extinction. In experiment 2, high IUS, relative to low IUS individuals were only found to show larger skin conductance responding to the learned cues with threatening properties during extinction i.e. mildly aversive tone. Bars represent standard error at + or - 1 SD of mean IUS. Square root transformed and z-scored SCR magnitude (μS), skin conductance magnitude measured in microSiemens.

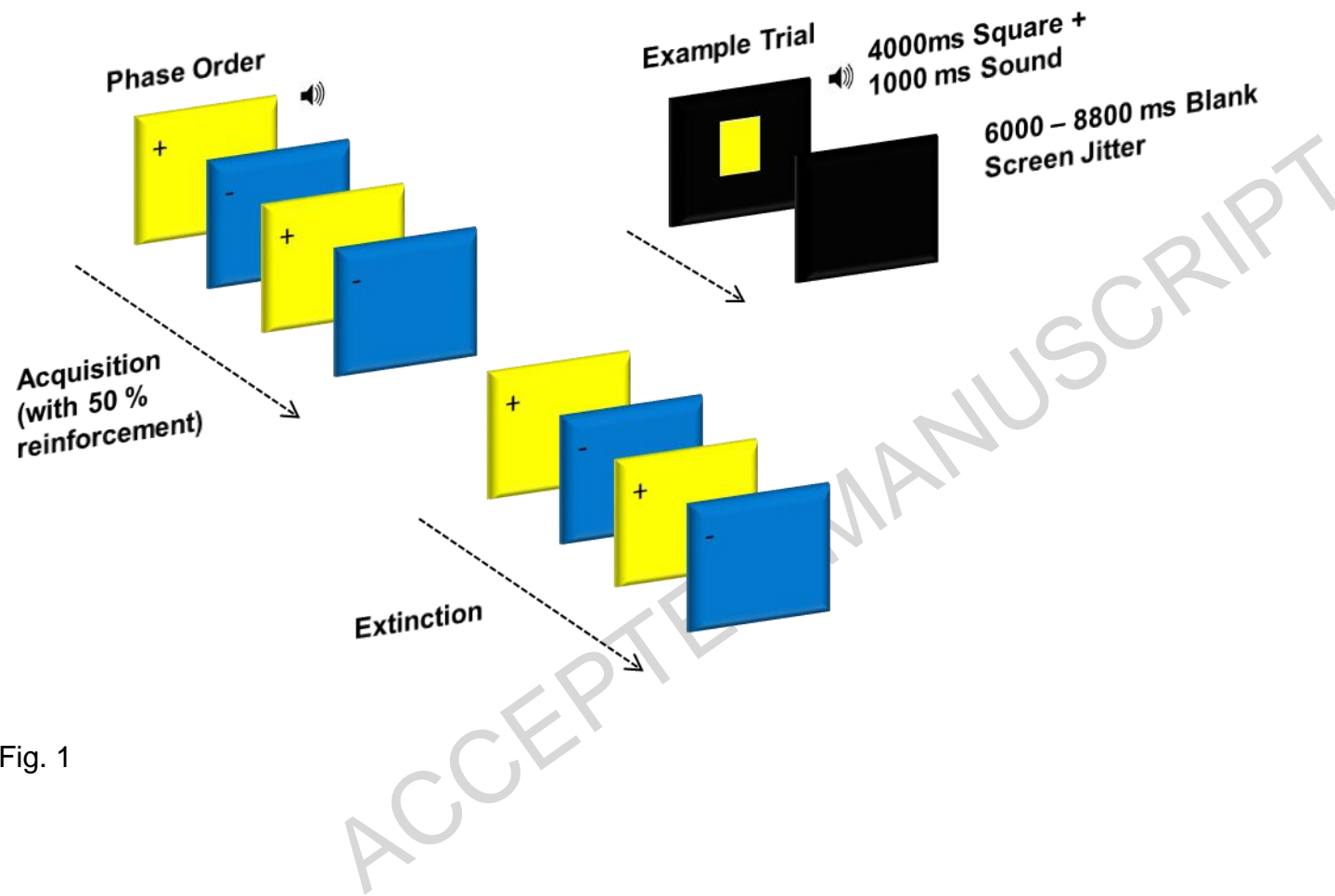


Fig. 1

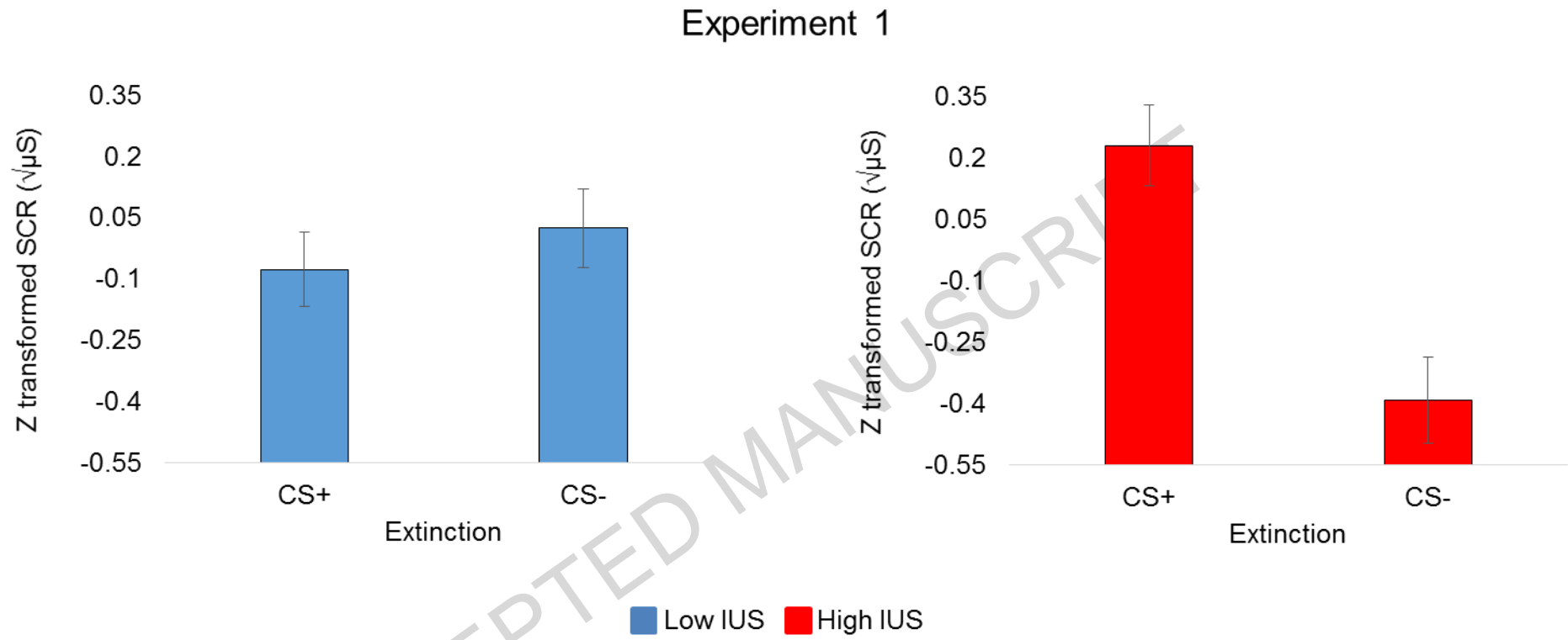


Fig. 2

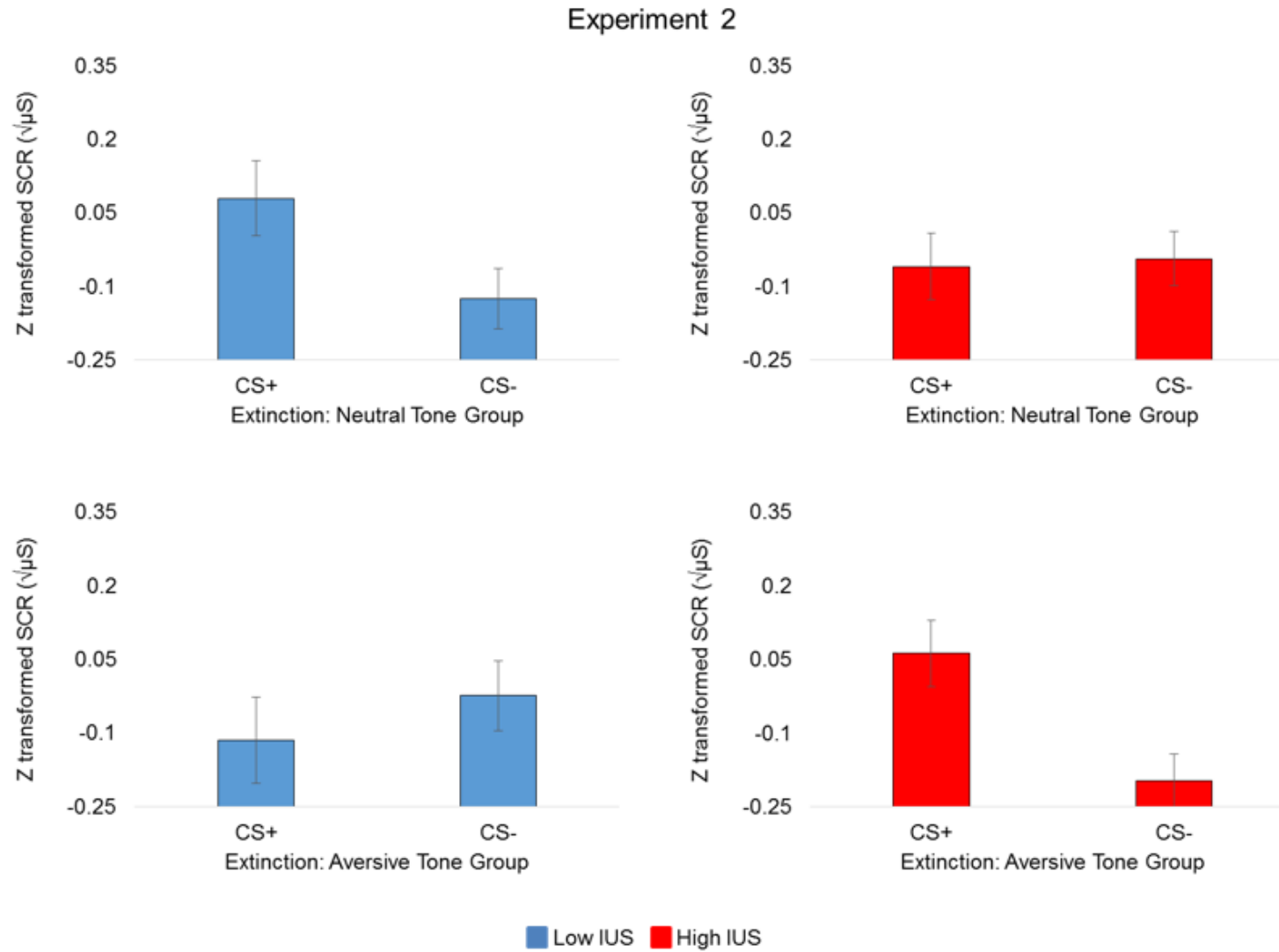


Fig. 3

Highlights

- We tested how threat level and Intolerance of Uncertainty (IU) impact extinction.
- We used skin conductance to index conditioned responding.
- High IU was associated with more responding to uncertain threat, even when mild.

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