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Personality Psychology

Shifty Eyes: The Impact of Intolerance of Uncertainty on Gaze Behaviour During Threat Conditioning

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Previous research has demonstrated that individuals with high levels of Intolerance of Uncertainty (IU) have difficulty updating threat associations to safety associations. Notably, prior research has focused on measuring IU-related differences in threat and safety learning using arousal-based measures such as skin conductance response. Here we assessed whether IU-related differences in threat and safety learning could be captured using eye-tracking metrics linked with gaze behaviours such as dwelling and scanning. Participants (N = 144) completed self-report questionnaires assessing levels of IU and trait anxiety. Eye movements were then recorded during each conditioning phase: acquisition, extinction learning, and extinction retention. Fixation count and fixation duration served as indices of conditioned responding. Patterns of threat and safety learning typically reported for physiology and self-report were observed for the fixation count and fixation duration metrics during acquisition and to some extent in extinction learning, but not for extinction retention. There was little evidence for specific associations between IU and disrupted safety learning (e.g., greater differential responses to the threat vs. safe cues during extinction learning and retention). While there was tentative evidence that IU was associated with shorter fixation durations (e.g., scanning) to threat vs. safe cues during extinction retention, this effect did not remain after controlling for trait anxiety. IU and trait anxiety similarly predicted greater fixation count and shorter fixation durations overall during extinction learning, and greater fixation count overall during extinction retention. IU further predicted shorter fixation durations overall during extinction retention. However, the only IU-based effect that remained significant after controlling for trait anxiety was that of fixation duration overall during threat extinction learning. Our results inform models of anxiety, particularly in relation to how individual differences modulate gaze behaviour during threat conditioning.

Introduction

The ability to learn and update information in response to threat and safety is a cornerstone of adaptive behaviour (Carpenter et al., 2019; Pittig et al., 2018). *Classical threat conditioning* paradigms are thought to model processes related to pathological fear and anxiety, as well as crucial principles underlying exposure-based therapies for anxiety and stress disorders (Craske et al., 2014). Threat conditioning paradigms typically include an *acquisition* phase, in which a neutral conditioned stimulus (CS+: e.g., visual cue) is reinforced with an aversive stimulus (unconditioned stimulus, US: e.g., electric shock). After several pairings, the CS+ becomes a signal for threat and its presentation alone elicits conditioned responses (CRs: e.g., ratings of fear and skin conductance responses). A second stimulus (CS-) is typically presented in the absence of the US to signal safety. An *extinction learning* phase follows and involves repeated exposure to the unreinforced CS+, which eventually leads to diminished CRs (Bouton, 2002; LeDoux, 1998). Evidence of spontaneous recovery (re-emergence of CRs to the CS+), assessed following a temporal delay in a subsequent *extinction retention* phase, suggests that extinction does not erase the previously acquired threat association, but instead represents active and adaptive learning of a new association by assigning a new value of safety to the cue

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that previously signalled threat (Bouton, 2002; Phan & Sripada, 2013). This new association of safety therefore competes for expression with the initially acquired threat association. Successful extinction learning and retention are therefore indexed by reduced CRs to the CS+, at levels comparable to CS- responding (Lonsdorf et al., 2017; Maren & Quirk, 2004). Conversely, continued differential CRs to CS+ relative to CS-, or exaggerated indiscriminate CRs to both the CS+ and CS-, are respectively indicative of retrieval of the initially acquired threat association, or of maladaptive generalisation of threat expression to learned threat and safety cues alike (Duits et al., 2015).

Uncertainty (also commonly referred to as ambiguity) about threat and safety contingencies may disrupt the learning and retention of new safety associations (Bouton, 2002; Levy & Schiller, 2021). Notably, recent work has highlighted the importance of individual differences in *Intolerance of Uncertainty* (IU: the tendency to hold negative beliefs about uncertainty) (Birrell et al., 2011; Carleton, 2016a, 2016b; Dugas et al., 2004), a transdiagnostic risk factor for anxiety and stress disorders (McEvoy et al., 2019), in modulating safety learning processes (Lonsdorf & Merz, 2017; Tanovic et al., 2018). Within the threat conditioning literature, prior research has demonstrated that higher levels of self-reported IU are typically associated with disrupted extinction learning and retention: during extinction learning, particularly during later trials in the extinction learning phase, individuals with higher levels of IU show greater CRs to the CS+ vs. CS-, as indexed by several psychophysiological and neural readout measures, such as skin conductance responses (SCRs), corrugator supercilii activity, and amygdala activity (Morriss, Wake, et al., 2021; Morriss, Zuj, et al., 2021). Higher levels of self-reported IU are additionally associated with greater SCRs in response to the CS+ vs CS- during extinction retention (Dunsmoor et al., 2015; Lucas et al., 2018; Wake et al., 2021), indicating that individuals with high IU have difficulty retaining new associations of safety following temporal delays. The effect of IU during extinction has consistently been demonstrated over and above broader measures of negative affective disposition such as trait anxiety (Morriss, Wake, et al., 2021; Morriss, Zuj, et al., 2021). IU is thought to maintain conditioned responses during extinction learning and retention because of the uncertainty experienced during these contexts (Morriss, Zuj, et al., 2021). Crucially, during extinction learning and retention there is uncertainty regarding the contingencies, as the probabilistic structure of the environment changes (threat cues become safe cues), and these changes are not always obvious in the first instance (Levy & Schiller, 2021; Morriss, Zuj, et al., 2021). Thus, during extinction learning and retention, individuals with high IU may find the uncertainty regarding the contingencies aversive, which subsequently disrupts and prolongs the learning or retention of new safety associations.

Despite advancements in understanding the involvement of IU in threat conditioning mechanisms, the majority of IU and conditioning literature has focused on using SCRs to assess conditioned responses. Although the use of SCRs has its strengths, as SCRs are generally discriminable and directly represent sympathetic activity (Dawson et al., 2007; Society for Psychophysiological Research Ad Hoc Committee on Electrodermal Measures, 2012), there are some drawbacks. For example, SCRs have a slow temporal resolution (Dawson et al., 2007) and individuals vary widely in the extent to which they consistently generate SCRs to even threat-relevant stimuli (Lonsdorf et al., 2017). Given this, further research on how IU modulates other metrics of conditioned responding is required to ascertain the reliability of IU-related effects during threat conditioning across measures (Lonsdorf et al., 2017; Morriss, Zuj, et al., 2021) and to understand whether IU may impact other relevant processes during threat conditioning that are related to pathological anxiety, such as attentional engagement (Tanovic et al., 2018).

In the context of threat conditioning, eye-tracking has several advantages over SCR. For instance, eye movements are quicker than SCRs and provide additional information regarding attentional engagement during threat conditioning. The bulk of eye-tracking studies have used visual search tasks to isolate eye movements and gaze to the CS+ relative to the CS- or other neutral competing stimuli (Koenig et al., 2017; Nissens et al., 2017; Onnis et al., 2011). Excitingly, several recent eye-tracking studies have demonstrated that gaze can be used as an index of conditioned responding when the CS+ and CS- are presented alone in the centre of a computer screen (Michalska et al., 2017; Xia et al., 2020). For instance, Xia et al. (2020) observed longer fixation duration (the length of time for which the eye pauses) and reduced fixation count (the number of discrete pauses of the eyes) to the CS+ relative to the CS- during threat acquisition, and no difference in fixation duration or fixation count to the CS+ vs. CS- during threat extinction learning. Notably, the eye-tracking metrics of fixation duration and fixation count can be linked to different gaze behaviours such as dwelling (e.g., longer fixation and smaller fixation count) and scanning (e.g., shorter fixation duration and larger fixation count) (Richards et al., 2014). Emerging research suggests that IU is associated with altered attentional processing, particularly for uncertain stimuli (Fergus et al., 2013), and eye-tracking studies have demonstrated effects of self-reported IU over trait anxiety on attention allocation towards uncertain information (Morriss et al., 2017; Morriss & McSorley, 2019). Nevertheless, to our knowledge, no research to date has examined how IU affects gaze behaviours during threat learning and extinction.

In this study, we analysed eye-tracking data that were collected but not analysed during a previously published conditioning and extinction study (Morriss et al., 2020). We used the eye-tracking data to investigate how IU was associated with gaze behaviours during threat acquisition, extinction learning and extinction retention. As in previous work (Morriss et al., 2019; Morriss & van Reekum, 2019), monochromatic coloured squares were presented as CSs, which were reinforced with an unpleasant sound (US) 50% of the time in acquisition. This was followed by extinction learning (same-day extinction, SDE) and extinction retention (next-day extinction, NDE) phases, in which the CSs were presented on their own, without the US. We focused

our analyses on fixation count and fixation duration to examine gaze behaviours such as dwelling and scanning.

The analyses aimed to (1) replicate existing findings on gaze in threat acquisition and extinction learning (Michalska et al., 2017; Xia et al., 2020), and on the effects of IU on arousal-based metrics in threat extinction learning and retention (Morriss, Wake, et al., 2021; Morriss, Zuj, et al., 2021), and (2) to extend these findings by examining whether individual differences in IU are related to differential gaze behaviours. Based on the evidence reviewed above, we hypothesised that, during acquisition, conditioned responses (CRs) would be observed, indexed by (1) fewer fixations and (2) longer fixation duration to the CS+ relative to the CS-. In addition, we hypothesised that CRs would dissipate throughout the extinction learning and retention phases, with no differential responses to the CS+ vs. CS- by late same-day extinction (SDE) and late next-day extinction (NDE). Crucially, we examined whether individual differences in IU are related to threat acquisition, extinction learning (SDE), and extinction retention (NDE), as indexed by gaze. Given prior mixed findings regarding IU and physiological responses during the acquisition phase (see review by Morriss, Zuj, et al., 2021; c.f. Mertens et al., 2022; Sjouwerman et al., 2020; Starita et al., 2019), we hypothesised that IU may be related to differences in fixation count and fixation duration to the CSs during acquisition, though we did not specify a direction of potential IU-based effects. Based on previous research on gaze in conditioning (Michalska et al., 2017; Xia et al., 2020), as well as existing research on IU in extinction (Morriss, Wake, et al., 2021; Morriss, Zuj, et al., 2021), we hypothesised that higher levels of self-reported IU, compared to lower levels of self-reported IU, would be related to continued CRs during late SDE and early NDE, as indexed by (1) fewer fixations and (2) longer fixation duration towards the CS+ relative to the CS-. Reflecting our prior research on IU, we assessed the specificity of IU on gaze behaviour by controlling for trait anxiety (Klingelhöfer-Jens et al., 2022; Mertens & Morriss, 2021; Sjouwerman et al., 2020).

Method

Participants

For the original study (Morriss et al., 2020), a sample of 144 participants aged 18 – 35 years were recruited from the University of Reading local area. Six participants did not return for Day 2 testing. Seven participants were excluded from Day 1 and eleven were excluded from Day 2 due to poor data quality and optic artefacts (multiple trials with missing data/ failure to track pupil), resulting in a final sample of n = 125 with Day 1 and Day 2 data and a final sample of n = 137 with Day 1 data only (see Table 1 for demographic information). Participants received a total of £15 for their involvement in the study (£5 at end of Day 1 testing, and £10 at end of Day 2 testing). Ethical approval for this study was granted by the University of Reading Research Ethics Committee.

There are no agreed-upon methods for power and sample size calculations for MLMs (Peugh, 2010; Snijders, 2005). Therefore, we based an a-priori sample size calculation on a repeated-measures within factors design, which was estimated using G*Power (Faul et al., 2009). The following parameters were entered: f = .26, α = .05, β = .80, number of groups = 1, number of measurements = 4 (max. per phase, e.g., SDE: early/late & CS+/CS-). The total sample size suggested was n = 22. The effect size of f = .26 was derived from Experiment 2 of Xia et al.'s (2020) paper (converted from Hedges' g = .52), which assessed the effect of threat acquisition and extinction learning on eye-tracking with stimulus timings that most closely match the design of the current study (3s time-period before US onset).

Furthermore, as analyses of individual differences tend to have small-to-medium-sized effects (Berenbaum et al., 2008; Bredemeier & Berenbaum, 2008; Hong & Lee, 2015; Morriss et al., 2020), an additional a-priori sample size calculation was estimated for our investigation of individual differences in IU within eye-tracking, with a small-medium effect size of f = .13, correlation among repeated measures = .30, and all other parameters as reported above. The total sample size suggested was n = 115. Therefore, though we were overpowered for our main effects analyses, our sample size was more appropriate for analyses of individual differences in IU.

Overall Procedure

The study took place over two sessions, which were separated by approximately 24 hours. On Day 1, participants were informed about the experimental procedure and seated in the testing booth, where they provided informed consent and completed questionnaires (see below). Prior to visiting the lab, participants were asked to remove eye make-up to avoid issues with pupil identification during eye-tracking (Carter & Luke, 2020). Participants then had the eye-tracker mounted on their head, completed the eye-tracker calibration process, and were presented with the conditioning task (see below), while eye movements were recorded. Participants were instructed to attend to the squares and sounds, to remain as still as possible, and keep their head on the chinrest. On Day 2, participants received the same instructions to the day prior, and underwent the same computer and physiological setup as on Day 1. Each of the testing sessions were performed in a dark room and each lasted approximately 30 minutes.

Apparatus

Eye movements were recorded monocularly (right eye only) using a head-mounted Eyelink II eye-tracker and pupil-only tracking mode with a sampling rate of 500 Hz, spatial resolution (RMS) of < 0.01°, and temporal resolution of 4 ms (EyeLink II Manual, SR Research). Head movements were constrained with a chinrest at a viewing distance of 57 cm. Calibration was achieved using a standard three-point grid at the start of the experiment, and then validated using a different grid. Participants were allowed to begin the experiment once there was an average difference of < 0.5° between the actual eye position and that predicted from the calibration and validation. Visual stimuli were presented

Table 1. Participant Demographics

	Day 1 and Day 2	Day 1 Only
N	125	137
Age	*M* = 24.27, *SD* = 4.50, range = 18-35	*M* = 24.16, *SD* = 4.46, range = 18-35
Ethnicity		
White	76 (60.8%)	85 (62.04%)
Asian	28 (22.4%)	29 (21.17%)
Middle Eastern/Arab	3 (2.4%)	4 (2.92%)
Black	2 (1.6%)	2 (1.46%)
Mixed	2 (1.6%)	2 (1.46%)
Not specified	14 (11.2%)	15 (10.95%)
Sex		
Female	77 (61.6%)	82 (59.85%)
Male	46 (36.8%)	53 (38.53%)
Not specified	2 (1.6%)	2 (1.46%)
Sexual orientation		
Heterosexual	92 (73.6%)	102 (74.45%)
Sexual minorities (lesbian/ gay/ bisexual/ pansexual)	17 (13.6%)	18 (13.14%)
Not specified	16 (12.8%)	17 (12.41%)

at a 75 Hz refresh rate on a 22-inch colour monitor with a resolution of 800 x 600 pixels (Mitsubishi DiamondPro 2070SB). Auditory stimuli were presented using over-ear dynamic stereo headphones (HD 206, Sennheiser, Wendemark-Wenneboste, Germany).

Stimuli

The CSs were monochromatic squares (blue: RGB values 205, 236, 255 and yellow: RGB values 255, 255, 3) with 192 x 180-pixel dimensions and visual angles of 6.16° x 9.07°, presented on the centre of the screen and surrounded by a black background. The US was a female scream, which has been used in previous experiments (Morriss et al., 2015, 2019; Morriss & van Reekum, 2019). The volume of the sound (90 dB) was standardised across participants by using fixed volume settings on the presentation computer and verified by an audiometer held against the headphones prior to each session.

Conditioning Task

The conditioning task was designed and presented using E-Prime 2.0 (Psychology Software Tools Ltd., Pittsburgh, PA). The task comprised of three conditioning phases: threat acquisition, same-day extinction (SDE) and next-day extinction (NDE). There were 24 trials in the acquisition phase and 32 trials in both extinction phases (see Fig. 1), with two blocks for each phase (two blocks of 12 trials during acquisition and two blocks of 16 trials in extinction). Early extinction was defined as the first 8 CS+/CS- trials, and late extinction as the last 8 CS+/CS- trials for both SDE and NDE. During acquisition, one of the stimuli (blue or yellow square) (CS+) was paired with the aversive sound (CS-US) 50% of the time, whilst the other stimulus (yellow or blue square) was presented alone (CS-). During both same- and next-day extinction, both CSs were presented without the US.

Participants were not instructed on contingencies or informed about the number of CSs. Conditioning contingencies were counterbalanced across participants, and experimental trials were pseudo-randomised, with the first acquisition trial always being paired, and all subsequent trial types presented at random. A 50% reinforcement schedule was used to maximise unpredictability of the CS-US contingency (Morriss, Zuj, et al., 2021). CSs were always centred on the screen and presented for a total of 4000 ms. The US was presented for 1000 ms and co-terminated with the reinforced CS+. Following this, a blank black screen was presented for 6000-8800 ms (see Fig. 1). Following the end of each block, participants were asked to provide expectancy ratings and were also asked to rate the valence and arousal of the US at the end of the experiment on Day 1 (reported elsewhere, see Morriss et al., 2020). There were no breaks between the acquisition and SDE phases.

Questionnaires

Intolerance of Uncertainty Scale (IUS)

The IUS is a 27-item self-report measure of emotional, cognitive, and behavioural responses to uncertainty (Carleton et al., 2007; Freeston et al., 1994). The scale has excellent internal consistency, α = .91 (Freeston et al., 1994). For each of the items (e.g., *I always want to know what the future has in store for me* or *When it's time to act, uncertainty paralyses me*), participants are asked to rate how characteristic it is of them on a 5-point Likert scale, where 1 = *not at*

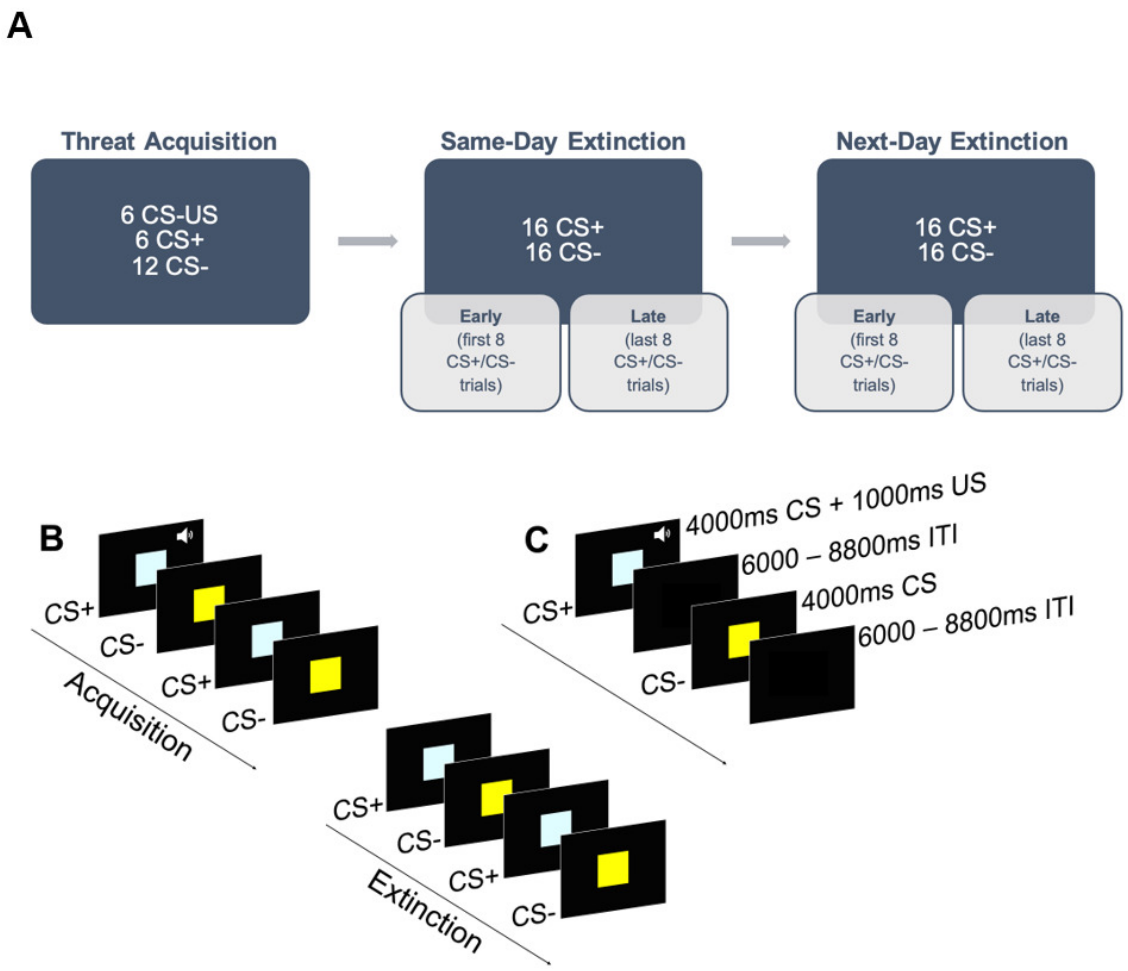

Figure 1. Image Depicting (A) Experimental Conditions and Procedure, (B) Acquisition (50% Reinforced) and Extinction Learning (SDE) and Extinction Retention (NDE) Phases, as well as (C) Example of Two Consecutive Trials.

Note. CS+ = reinforced conditioned stimulus; CS- = unreinforced conditioned stimulus; US = unconditioned stimulus; ITI = inter-trial-interval. The US co-terminated with the CS+. SDE and NDE were identical in terms of procedure.

all characteristic of me, and 5 = *entirely characteristic of me.* Total scores range from 27-135, with higher scores indicating higher levels of IU.

State-Trait Inventory for Cognitive and Somatic Anxiety (STICSA)

The STICSA (Ree et al., 2008) is a 21-item self-report measure of state and trait anxiety. The latter version was utilised for the purposes of this study to control for trait anxiety, as, contrastingly to other trait anxiety measures, which include depressive symptomology, the STICSA is a purer indicator of anxiety (Grös et al., 2007). The scale has excellent internal consistency, commonly > .87 (Grös et al., 2007). Participants are instructed to read each statement (e.g., *I feel agonised over my problems* or *My face feels hot*) and, using a 4-point Likert scale, indicate how often, in general, the statement is true of them, where 1 = *not at all*, and 4 = *very much so*. Total scores range from 21-84, with higher scores indicating higher levels of trait anxiety.

Preparation of Eye-Tracking Data

Raw eye-tracking data were automatically segmented online into sequences of saccades and fixations using the EyeLink II parser system, which identifies the start and end of saccades using 30°/s velocity, and 8000°/s^2 acceleration criteria (EyeLink II Manual, SR Research). Eye movements that did not meet criteria for saccades were defined as fixations, as is common in eye-tracking literature (Holmqvist & Andersson, 2017). As the stimuli were simple (monochromatic squares), regions of interests were not specified.

Data were then visually inspected for quality offline using DataViewer (version 4.2.1), at which point participant exclusions were identified. Participants were excluded if they met two or more of the following criteria: > 1 trial without fixations (indicative of potential issues recording the data/pupil not captured well), average blink sample count ⩾ 10% (indicative of samples across trials that were occluded in a blink), or average off-screen sample count of ⩾ 10% (indicative of samples across all trials that fell outside of the display boundary i.e. off-screen, and so may result from calibration or drift issues). Based on these crite-

ria, data of seven participants were excluded from Day 1 and eleven from Day 2.

Following this, fixation reports with variables of interest were generated. As overall attention engagement reflects gaze across total stimulus duration (Holmqvist et al., 2015), the following variables, which reveal individual interactions with stimuli on a global level (Carter & Luke, 2020), and most closely match the variables investigated by Michalska et al. (2017) and Xia et al. (2020), were quantified:

1. fixation count (number of discrete pauses of the eyes)
2. fixation duration (length of time for which the eye pauses (ms)).

Finally, the data were cleaned using R (version 4.0.2), as follows: fixations during CS paired trials were discarded to avoid confounds with the unconditioned stimulus (i.e., the aversive sound). Eye movements that crossed stimulus presentation boundaries, i.e., that began prior to stimulus onset, were excluded from analyses, and fixations that ended following stimulus offset were trimmed to end at the end of stimulus presentation. In order to obtain a complete overview of gaze, no further exclusion criteria were adopted. Eye movements were averaged across trials per stimulus type and conditioning phase for the full viewing period (4000 ms following CS onset), resulting in fixation count and fixation duration data for the following conditions: Acquisition CS+, Acquisition CS-, Early SDE CS+, Early SDE CS-, Late SDE CS+, Late SDE CS-, Early NDE CS+, Early NDE CS-, Late NDE CS+, Late NDE CS-.

Analysis Strategy

We conducted multilevel models (MLMs) in R version 4.0.2 using the *lmer* function from the *lme4* package (Bates et al., 2015). The raw data and analysis scripts are available at https://osf.io/985je/. To first establish whether successful threat acquisition and subsequent extinction effects were observed, separate MLMs were conducted for fixation count and fixation duration for i) acquisition, ii) SDE and iii) NDE. For the acquisition phase, Stimulus (CS+, CS-) was entered at level 1 and individual subjects at level 2. For the SDE and NDE phases, Stimulus (CS+, CS-) and Time (Early Extinction: first 8 CS+/CS- trials, Late Extinction: last 8 CS+/CS- trials) were entered at level 1 and individual subjects at level 2. Furthermore, the study in which the eye-tracking data were collected included an extended extinction condition with additional trials during SDE and NDE phases (32 trials for regular and 48 trials for extended extinction) (Morriss et al., 2020). As this was not the focus of the current study and we included only the first 32 SDE and NDE trials for all participants, we entered Condition (Regular, Extended) as a grouping factor at level 1 in the NDE MLMs to ascertain whether this would influence our findings.[1] Fixed effects included Stimulus and Time (and Condition for NDE), and random effects included a random intercept for each individual subject. A maximum likelihood estimator was utilised in all models. Level 1 variables were categorical and therefore effect coded (Stimulus: CS+ = 1, CS- = -1; Time: Early Extinction = 1, Late Extinction = -1; Condition: Extended = 1, Regular = -1).

We then carried out separate MLMs to investigate the effect of individual difference predictors IUS and STICSA, where grand-mean centred IUS and STICSA scores were included as continuous predictor variables in the MLMs, with all parameters as described above. Separate MLMs were initially carried out to investigate the effect of each individual difference predictor on dependent variables (i.e., separate models for IUS and STICSA). In the case of significant interactions observed with IUS or STICSA scores, follow-up MLMs were conducted with both IUS and STICSA scores included to assess specificity. A significant interaction with one of these predictors (IUS or STICSA) but not the other would indicate specificity of that predictor.[2] In line with previous work (Klingelhöfer-Jens et al., 2022; Mertens & Morriss, 2021), to further understand any significant IUS/STICSA effects or interactions from the MLMs, we conducted follow-up two-tailed correlations between the relevant self-report measure (IUS or STICSA) and dependent variable of interest (e.g. fixation count and fixation duration during a particular condition or phase).

Results

Questionnaires

As reported in the original paper (Morriss et al., 2020), the internal reliability of IUS and STICSA questionnaires was high (α = .95 [95% CI: 0.94, 0.96]; α = .88 [95% CI: 0.85, 0.90]). IUS was positively significantly correlated with STICSA [$r(144) = 0.68$, $p < .001$]. Data for both the IUS (M = 65.81, SD = 20.11, range = 32-125) and STICSA (M = 40.59, SD = 9.59, range = 22-69) were normally distributed (see Supplementary Materials).

Eye-Tracking Data

The results are presented by conditioning phase (acquisition, same day extinction, next day extinction) and by dependent variable (fixation duration, fixation count) below. In each section the first paragraph reports the results of the initial MLM analyses which included Stimulus, Time and Stimulus x Time interactions. These results are also visualised in Fig. 2 (see figure note for a summary of these effects). For a visualisation of eye-tracking data by trial, please see Supplementary Figure 8. The second paragraph of each section reports the results for the MLM analyses including the main effects of IUS, as well as Stimulus/Time x

1 As there were not any significant interactions observed between Stimulus x Condition, or between Stimulus x Condition x IUS/STICSA, we did not run any further analyses to examine the effects of this manipulation.

2 As there were not any significant effects or interactions observed with IUS or STICSA throughout acquisition, we did not run additional MLM analyses to examine specificity of IUS or STICSA during the acquisition phase.

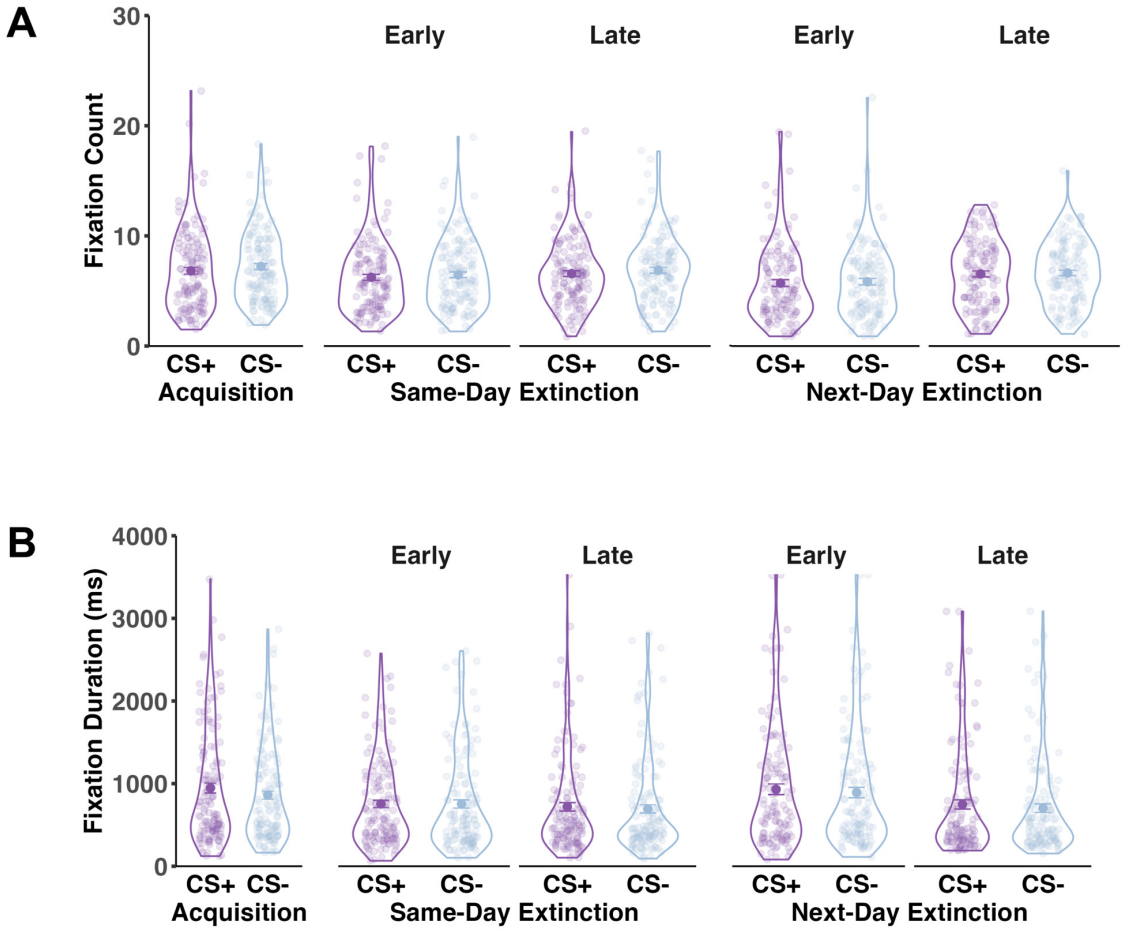

Figure 2. Violin Plots Depicting Main Effects of Stimulus and Time on (A) Fixation Count, and (B) Fixation Duration per Conditioning Phase.

Note. Individual datapoints presented here are fixation count and fixation duration averaged for each participant per stimulus, time and conditioning phase. Filled circles denote mean of fixation count and fixation duration per stimulus, time and conditioning phase. Error bars denote standard error of the mean. Early = first 8 CS+/CS- trials; Late = last 8 CS+/CS- trials. MLM analyses indicated that significantly fewer fixations for the CS+ vs CS- were observed during acquisition and SDE but not NDE, and there were significantly fewer fixations throughout both early SDE and NDE than late SDE and NDE (A). There were significantly longer fixations for the CS+ vs CS- during acquisition, and fixation duration did not significantly vary in response to CS+ vs CS- during SDE or NDE or across early or late SDE, but there were significantly longer fixations throughout early than late NDE (B).

IUS interactions. These results are also presented in Table 2 along with comparable models using STICSA. The STICSA models are presented in Table 2 for transparency but are not elaborated on in the text. Follow-up tests (i.e., MLMs with both IUS and STICSA assessing their specificity) are presented in the text only.

Acquisition

Fixation Count

As predicted, fixation count was significantly lower for the CS+ compared to the CS- during the acquisition phase [Stimulus: $F(1,137) = 10.72$, $p = .001$] (see Fig. 2a).

Reflecting prior work with arousal-based metrics, IUS was not a significant predictor of fixation count during acquisition [IUS: $F(1, 137) = 2.86$, $p = .093$], and individual differences in IUS were not significantly related to differential fixation counts in response to the CS+ vs CS- [Stimulus x IUS: $F(1,137) = 0.02$, $p = .897$].

Fixation Duration

As predicted, fixations towards the CS+ were of significantly longer duration relative to the CS- during acquisition [Stimulus: $F(1,137) = 8.73$, $p = .004$].

Again, IUS was not significantly associated with fixation duration during acquisition [IUS: $F(1, 137) = 3.42$, $p = .067$], and individual differences in IUS were not significantly related to differential fixation duration to the CS+ vs. CS- [Stimulus x IUS: $F(1, 137) = 0.14$, $p = .708$].

Same Day Extinction

Fixation Count

Contrary to predictions, the Stimulus x Time interaction for fixation count during SDE was not significant [Stimulus x Time: $F(1, 411 = 0.08$, $p = .773$). Instead, there was a significant main effect of Stimulus [Stimulus: $F(1, 411) = 3.95$, $p = .048$], indicating differential fixation counts in response to the CS+ (early and late) relative to the CS- (early and late) throughout SDE, with fewer fixations in response to

Table 2. IUS and STICSA Main Effects and Interactions from MLMs per Conditioning Phase and Eye-Tracking Variable.

	Acquisition		Same-Day Extinction		Next-Day Extinction	
	Fixation Count	**Fixation Duration**	**Fixation Count**	**Fixation Duration**	**Fixation Count**	**Fixation Duration**
IUS	*F(1, 137) = 2.86, p = .093*	*F(1, 137) = 3.42, p = .067*	F(1, 137) = 4.59, p = .034	**F(1, 137) = 8.22, p = .005**	F(1, 126.88) = 5.94, p = .016	F(1, 126.87) = 7.62, p = .007
STICSA	*F(1, 137) = 2.40, p = .124*	*F(1, 137) = 0.49, p = .483*	F(1, 137) = 5.16, p = .025	F(1, 137) = 4.17, p = .043	F(1, 126.76) = 4.22, p = .042	*F(1, 126.82) = 3.57, p = .061*
Stimulus x IUS	*F(1, 137) = 0.02, p = .897*	*F(1, 137) = 0.14, p = .708*	*F(1, 411) = 1.15, p = .284*	*F(1, 411) = 1.73, p = .189*	F(1, 379.21) = 4.05, p = .045	*F(1, 379.24) = 1.50, p = .222*
Stimulus x STICSA	*F(1, 137) = 0.77, p = .381*	*F(1, 137) = 0.12, p = .734*	*F(1, 411) = 0.02, p = .880*	*F(1, 411) = 0.46, p = .496*	*F(1, 378.95) = 2.68, p = .103*	*F(1, 379.03) = 0.93, p = .336*
Time x IUS	-	-	*F(1, 411) = 3.29, p = .070*	*F(1, 411) = 0.03, p = .861*	*F(1, 378.97) = 0.34, p = .559*	*F(1, 378.97) = 1.98, p = .160*
Time x STICSA	-	-	*F(1, 411) = 1.32, p = .251*	*F(1, 411) = 0.08, p = .777*	*F(1, 378.93) = 0.47, p = .494*	*F(1, 379.01) = 2.12, p = .146*
Stimulus x Time x IUS	-	-	*F(1, 411) = 1.47, p = .227*	*F(1, 411) = 0.13, p = .722*	*F(1, 378.97) = 2.55, p = .111*	*F(1, 378.97) = 1.47, p = .226*
Stimulus x Time x STICSA	-	-	*F(1, 411) = 1.19, p = .276*	*F(1, 411) = 0.06, p = .802*	*F(1, 378.93) = 2.34, p = .127*	*F(1, 379.01) = 2.25, p = .134*

Note. Entries in the table that are formatted in bold indicate $p < .05$ and that the effect was significant when controlling for IUS or STICSA. Black font indicates $p < .05$ and that the effect was not significant when controlling for IUS or STICSA. Entries in the table that are italicised denote $p > .05$.

the CS+ relative to the CS-. There was additionally a significant main effect of Time [Time: F (1, 411) = 7.74, p = .006] on fixation count throughout SDE, whereby there were fewer fixations throughout early SDE when compared to late SDE (see Fig. 2a).

A significant effect of IUS on fixation count in SDE was observed when IUS was entered into the model alone (p = .034, see Table 2), with higher levels of IUS being associated with a higher number of fixations across SDE (See Supplementary Materials). However, the effect of IUS was no longer significant when entered into the model together with STICSA, [IUS: F(1, 137) = 0.64, p = .426; STICSA: F(1, 137) = 1.19, p = .278]. IUS was not significantly associated with differential fixation counts towards the CS+ vs the CS- during SDE [Stimulus x Time x IUS: F(1, 411) = 1.47, p = .227; Stimulus x IUS: F(1, 411) = 1.15, p = .284] or fixation counts across time during SDE [Time x IUS: F(1, 411) = 3.29, p = .070].

Fixation Duration

There were no significant differences in fixation duration in response to the CS+ vs. the CS- throughout SDE [Stimulus: F(1, 411) = 0.25, p = .617], and fixation durations did not vary significantly as a function of time [Time: F(1,411) = 2.96, p = .086; Stimulus x Time: F(1, 411) = 0.21, p = .650].

During SDE, there was a significant effect of IUS both when IUS was entered into the model alone [p = .005, see Table 2], and when entered with STICSA [IUS: F(1, 137) = 3.95, p = .049; STICSA: F(1, 137) = 0.01, p = .906]. A follow-up correlational test showed that higher IUS was significantly associated with shorter fixation durations across the SDE phase [r (137) = -0.24, p = .005] (see Fig. 3). IUS was not significantly associated with differential fixation duration to the CS+ vs the CS- during SDE [Stimulus x Time x IUS: F(1, 411) = 0.13, p = .722; Stimulus x IUS: F(1, 411) = 1.73, p = .189] or fixation duration across time during SDE [Time x IUS: F(1, 411) = 0.03, p = .861].

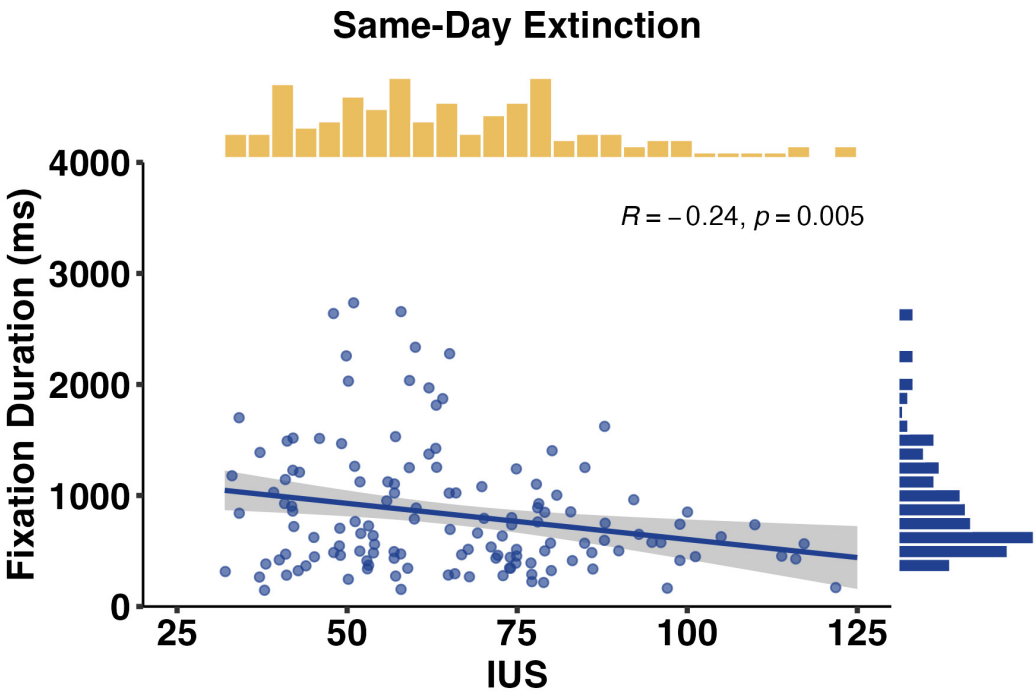

Figure 3. Scatterplot With Histogram Depicting Correlation Between IUS and Fixation Duration Throughout the SDE Phase.

Note. The distribution of IUS scores is displayed on top of the figure in yellow, and the distribution of SDE fixation duration is displayed on the right side of the figure in blue. Shaded areas represent 95% confidence intervals. Higher IUS was associated with shorter fixation durations throughout SDE.

Next Day Extinction

Fixation Count

There were no significant differences in fixation count in response to the CS+ vs. the CS- throughout NDE [Stimulus: $F(1, 379.40) = 0.36$, $p = .547$] (see Fig. 2a). Even though there were significantly fewer fixations in early compared to late NDE [Time $F(1, 378.95) = 28.96$, $p < .001$], the number of fixations in response to the CS+ vs the CS- did not vary significantly as a function of time [Stimulus x Time: $F(1,378.95) = 0.01$, $p = .958$].

There was a significant Stimulus x IUS interaction and significant main effect of IUS when IUS was entered into the model alone [Stimulus x IUS $p = .045$; IUS $p = .016$, see Table 2), with higher levels of IUS being associated with a higher number of fixations overall and towards the CS+ across NDE (see Supplementary Materials). However, this interaction and main effect did not remain significant when both IUS and STICSA were entered together in the model [Stimulus x IUS: $F(1, 379.39) = 1.52$, $p = .218$; IUS: $F(1, 126.89) = 3.03$, $p = .084$; STICSA: $F(1, 126.83) = 0.01$, $p = .907$]. No other significant interactions with stimulus or time emerged for IUS [Stimulus x Time x IUS: $F(1, 378.97) = 2.55$, $p = .111$; Time x IUS: $F(1, 378.97) = 0.34$, $p = .559$].

Fixation Duration

Reflecting the findings for fixation count, there was not a main effect of Stimulus on fixation duration throughout NDE [Stimulus: $F(1, 379.50) = 2.11$, $p = .147$], which indicated there were no significant differential fixation durations in response to the CS+ (early and late) relative to the CS- (early and late) throughout this phase (see Fig. 2b). Though fixation durations in response to both stimuli were significantly longer in early compared to late NDE [Time: $F(1, 379.02) = 31.65$, $p < .001$], fixation durations towards the CS+ vs the CS- did not vary as a function of time [Stimulus x Time: $F(1, 379.02) = 0.01$, $p = .935$].

IUS was significantly associated with fixation durations across NDE when IUS was entered into the model alone [$p = .007$, see Table 2]. However, the effect of IUS was no longer significant when entered into the model together with STICSA [IUS: $F(1, 126.89) = 3.03$, $p = .084$; STICSA: $F(1, 126.83) = 0.01$, $p = .907$]. No other significant interactions emerged for IUS throughout NDE [Stimulus x Time x IUS: $F(1, 378.97) = 1.47$, $p = .226$; Stimulus x IUS: $F(1, 379.24) = 1.50$, $p = .222$; Time x IUS: $F(1, 378.97) = 1.98$, $p = .160$].

Discussion

Here we utilised eye-tracking in a conditioning paradigm with threat acquisition, extinction learning, and extinction retention phases to examine the effect of individual differences in IU on threat and safety learning. Fixation count and fixation duration were used as indices of conditioned responding. We generally replicated previous work demonstrating the utility of eye-tracking metrics related to gaze in threat acquisition and extinction learning (Michalska et al., 2017; Xia et al., 2020), as well as extended our understanding of how these eye-tracking metrics operate during extinction retention. We failed to replicate previous research demonstrating specific associations between IU and poorer

safety learning (e.g., greater differential responses to the CS+ vs. CS- during extinction learning and retention) (Morriss, Wake, et al., 2021; Morriss, Zuj, et al., 2021). While IU was significantly associated with greater fixation count (e.g., scanning) to the CS+ vs. CS- during extinction retention, this effect did not hold when controlling for trait anxiety. Both IU and trait anxiety similarly predicted greater fixation count and shorter fixation durations (e.g., scanning) overall during extinction learning, and greater fixation count overall during extinction retention. IU also predicted shorter fixation durations overall during extinction retention. However, the only IU-based effect that remained significant after controlling for trait anxiety was that of fixation duration overall during threat extinction learning. These results further our understanding of the role of IU in gaze behaviours during extinction learning and retention and inform models of attention- and exposure-based therapies.

During acquisition, successful threat conditioning was observed, with fewer but longer fixations in response to the CS+ relative to the CS-. Further, across SDE, our results indicate continued conditioned responding in terms of fixation count (indexed by continued differential fixation counts in response to CS+ vs CS-) but not in terms of fixation duration (no differential fixation durations to CS+ vs. CS-). During NDE, no differential fixation count or fixation duration to the CS+ vs. CS- was observed. However, during NDE, there were fewer fixations with a longer duration in the early half of NDE, compared to the late half of NDE. In relation to the original study (Morriss et al., 2020), the general effects of conditioning on eye gaze here are similar to what was observed for skin conductance response. However, the effects of eye gaze seem less reliable for extinction retention, compared to skin conductance response. In summary, these findings generally suggest that gaze behaviours such as dwelling are increased for the CS+ vs. CS- during acquisition and extinction learning. Importantly, these findings replicate patterns of fixation count and fixation duration found in prior acquisition and extinction learning research (Michalska et al., 2017; Xia et al., 2020). Our results are also in line with the wider literature that has demonstrated greater attention allocation to threat in general (Armstrong & Olatunji, 2012; Bar-Haim et al., 2007; Cisler & Koster, 2010).

IU was not observed to significantly impact differential fixation count and fixation duration to the CS+ vs. CS- during threat acquisition or extinction learning. However, IU was significantly associated with greater fixation count (e.g., scanning) to the CS+ vs. CS- during extinction retention, although this effect did not hold when controlling for trait anxiety. Interestingly, both IU and trait anxiety similarly predicted greater fixation count and shorter fixation durations (e.g., scanning) overall during threat extinction learning, and greater fixation count overall during threat extinction retention. Furthermore, IU predicted shorter fixation durations overall during extinction retention. However, the only IU-based effect that remained significant after controlling for trait anxiety was that of fixation duration overall during threat extinction learning. The lack of specificity of IU over trait anxiety is surprising, given that the majority of the literature has demonstrated that IU, controlling for trait anxiety, is associated with greater differential physiological responding (e.g. skin conductance, corrugator supercilii) to the CS+ vs. CS- during threat extinction learning and retention (Bauer et al., 2020; Morriss, 2019; Morriss, Christakou, et al., 2016; Morriss et al., 2015, 2019; Morriss, Macdonald, et al., 2016; Morriss & van Reekum, 2019; Wake et al., 2021). The discrepancy in the pattern of results for eye-tracking metrics related to gaze compared to other physiological measures such as skin conductance response and corrugator supercilii activity may reflect how IU and trait-anxiety-related biases alter different (but overlapping) physiological response systems. Moreover, it is possible that relationships between eye-tracking metrics related to gaze during threat conditioning and individual differences result in smaller effect sizes, compared to other physiological measures such as skin conductance response. Thus, IU-based specificity over trait anxiety may have been observed if the sample was larger.

Limitations and Future Directions

The study did have a few limitations. Firstly, we used calibration and validation procedures at the start of the experiment, but we did not implement these procedures throughout the experiment, which would have further improved data quality (Carter & Luke, 2020). Secondly, future work may additionally refine our findings by investigating spatially and temporally specific eye movements and gaze related to particular stimulus interest areas. These may ascertain the time-course of attentional engagement by indexing initial orienting (Mogg & Bradley, 2016), attention maintenance (Koster et al., 2004), and active avoidance behaviours (Pflugshaupt et al., 2007). Thirdly, although we found effects of conditioning, the experimental design used here (e.g. simple geometric shapes in the centre of the screen) may not be ideal for assessing gaze behaviour. Further work may wish to change the experimental design (e.g. use conditioned stimuli that are more dynamic - move around the computer screen; Nissens et al., 2017) to fully optimise capturing gaze behaviour during conditioning.

Conclusion

In sum, there was little evidence for specific associations between IU and poorer safety learning (e.g., greater differential responses to the CS+ vs. CS- during extinction learning and retention) (Morriss, Wake, et al., 2021; Morriss, Zuj, et al., 2021). While there was tentative evidence that IU was associated with shorter fixation durations to CS+ vs. CS- during extinction retention, this effect did not remain after controlling for trait anxiety. Both IU and trait anxiety similarly predicted greater fixation count and shorter fixation durations (e.g., scanning) overall during extinction learning, and greater fixation count overall during extinction retention. Additionally, IU predicted shorter fixation durations overall during extinction retention. However, the only IU-based effect that remained significant after controlling for trait anxiety was that of fixation duration overall during

threat extinction learning. Future studies should use more spatially and temporally specific eye movement and gaze metrics to improve our understanding of how IU alters attentional processes during extinction learning and retention. Hyper scanning may contribute to difficulties updating threat to safety, and thus may be a useful behaviour to target within exposure-based therapies for anxiety and stress disorders.

Author Contributions

S.W., H.D., & J.M. conceived and developed the ideas for this research. J.M. and H.D. obtained funding. S.W. and J.M. collected the data. E.M. helped with eye-movement data collection and extraction protocols. C.R-S. conducted the data reduction, statistical analyses and interpretation and wrote the manuscript draft. S.W., C.v.R. & J.M. advised on all stages of the data reduction and analysis. S.W., H.D., E.M., C.v.R & J.M. contributed to interpretation and critical manuscript revision and editing. All authors approved the final manuscript.

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Competing Interests

The authors declare no conflicts of interest.

Ethics Approval

This study was performed in line with the principles of the Declaration of Helsinki. Approval was granted by the University of Reading Research Ethics Committee.

Consent to Participate

Informed written consent was obtained from all participants included in the study.

Supplementary Materials

Supplementary data to this article can be found at https://osf.io/985je/.

Availability of data and materials

All data have been made publicly available via OSF and can be accessed at https://osf.io/985je/. The hypotheses and analyses reported here were not preregistered. Data from the original study can be accessed at https://osf.io/2ugpv/. The design and analysis plan for the original study was preregistered and can be accessed at https://osf.io/2ugpv/.

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