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Eye want to be sure: establishing the role of intolerance of uncertainty in eye-tracking conditioning paradigms

SEPT 2021

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Dr Carien van Reekum & Dr Jayne Morriss

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ABSTRACT

Background: High levels of Intolerance of Uncertainty (IU) cause individuals to either avoid or seek out disproportionate amounts of information in uncertain situations. Here we assessed attentional bias and ascertained hyper-vigilant or hyper-scanning behaviours by using eye-tracking - a direct measure of visual information seeking. This was measured in the context of a conditioning paradigm, with threat acquisition and extinction phases, as there is a lot of uncertainty involved in extinction. ***Objectives:*** We aimed to replicate existing but separate findings in eye-tracking and IU conditioning paradigms, and to apply this methodology in the context of IU. We also sought to ascertain whether effects would be specific to IU or could be attributed to trait anxiety. ***Methods:*** Participants (n = 144) completed self-report questionnaires that assessed levels of IU and trait anxiety. We then recorded eye-movements during threat acquisition and extinction. Fixation count, fixation duration, and saccade amplitude were quantified from eye-movements as indices of global attention and conditioned responses. ***Results:*** We replicated previous work demonstrating that eye-tracking is a viable index of conditioned responses in threat acquisition and extinction. We also found that this methodology can be applied in the context of IU, as high and low IU individuals demonstrated differential patterns of eye-movements. These effects were attributed to individual differences in IU over trait anxiety in extinction, but not in acquisition. ***Conclusions:*** These findings not only further our understanding of the role of IU and its psychophysiological basis in conditioning paradigms, but also demonstrate that eye-tracking can be added to the toolbox of methodologies utilised to study IU in threat acquisition and extinction. Our results inform models of IU, trait anxiety, and exposure-based therapies.

Key words: intolerance of uncertainty · trait anxiety · conditioning · threat acquisition · extinction · attentional bias · hyper-vigilance · hyper-scanning · eye-tracking · fixation count · fixation duration · saccade amplitude

1. INTRODUCTION

The ability to learn and update information in response to threat and safety is a cornerstone of adaptive behaviour and effective decision-making (Carpenter et al., 2019; Pittig et al., 2018). Classical conditioning is often thought to be an etiological model of pathological fear and anxiety (Ojala & Bach, 2020), as well as of exposure therapies that are used to treat them (Craske et al., 2014). Recent research has begun to highlight the importance of individual differences in *Intolerance of Uncertainty* (IU) (Freeston et al., 1994), the dispositional tendency to hold negative beliefs about uncertainty and its implications (Carleton et al., 2007; Dugas et al., 2004), in conditioning paradigms (Lonsdorf & Merz, 2017). Given growing evidence of its ubiquity (Berenbaum et al., 2008; Norton, 2005; Sexton & Dugas, 2009) and transdiagnostic role in mental health disorders (Carleton et al., 2012; McEvoy & Mahoney, 2012), conceptual understanding of IU, its psychophysiological basis, and how this may inform exposure-based treatments has become essential.

When faced with uncertainty, individuals with high levels of IU adopt maladaptive behaviours that modulate attention such as avoidance or disproportionate information-seeking (Buhr & Dugas, 2002; Carleton et al., 2012; Dugas et al., 2004). We therefore sought to assess such behaviours by using a direct measure of visual information seeking and global attention: eye-tracking. As there is a lot of uncertainty involved in conditioning, specifically in threat extinction (Levy & Schiller, 2021), we measured these behaviours in the context of a conditioning paradigm with threat acquisition and extinction phases.

The literature review comprises of four sections. The first section provides a broad overview of intolerance of uncertainty and its conceptualisation. This is followed by a review of existing literature on the role of intolerance of uncertainty in threat acquisition and extinction. The third section considers the nature of attentional bias and is dedicated to existing research on the use of eye-tracking in conditioning paradigms. Finally, in the fourth section, fundamental ideas concerning a connection between attention, eye-tracking, and intolerance of uncertainty are proposed, and the rationale for introducing eye-tracking to research on intolerance of uncertainty is outlined.

1.1 Intolerance of Uncertainty

- What do we know about IU?
- How does it differ from anxiety?
- Carleton 2016.
- Subscales?

Recent research has shed light on the conceptual nature of IU, and increasingly robust evidence suggests that IU comprises of two dimensions: *prospective* IU (P-IU), and *inhibitory* IU (I-

IU), which are thought to reflect a desire for predictability and uncertainty paralysis, respectively (Carleton, 2012; Carleton, Sharpe & Asmundson, 2007; Hong & Cheung, 2015; McEvoy & Mahoney, 2011; Berenbaum, Bredemeier & Thompson, 2008; Birrell, Meares, Wilkinson & Freeston, 2011). These dimensions are often associated with opposing psychophysiological responses (Jackson et al., 2016; Nelson et al., 2015), and have been differentially associated with mental health disorders, with P-IU sharing stronger associations with worry and symptoms of obsessive-compulsive disorder, and I-IU uniquely linked to symptoms of social anxiety, panic disorder, agoraphobia, posttraumatic stress disorder, and depression (Berenbaum et al., 2008; Carleton, 2012; Fetzner et al., 2013; Helsen et al., 2013; McEvoy & Mahoney, 2011,2012, 2012; Sexton & Dugas, 2009). Different aspects of IU may therefore drive different psychophysiological responses or behaviours when faced with uncertainty.

1.2 Intolerance of Uncertainty and Conditioning Paradigms

Classical conditioning paradigms typically include a *threat acquisition* phase, in which a neutral conditioned stimulus (CS+) is reinforced with an aversive stimulus (unconditioned stimulus, US). After several pairings, the CS+ becomes a signal for threat, and presentation of the CS+ alone elicits conditioned responses (CRs), indicating successful threat acquisition and characterising the development of a learned fear (Mineka & Zinbarg, 2006). CRs are measured physiologically through changes in sympathetic arousal of the autonomic nervous system, which are typically indexed by elevated skin conductance responses (SCRs), corrugator supercilii activity, and pupil dilation (Dunsmoor & LaBar in Armony & Vuilleumier 2013; Harisson, Kreibig & Critchely in Armory & Vuileumier, 2013, LeDoux, 1996). A discrimination variant of the CS+ is also introduced (CS-) to provide a baseline for comparison (Phelps & LeDoux, 2005).

A *threat extinction* phase follows threat acquisition and involves repeated exposure to the unreinforced CS+, which eventually leads to diminished CRs. Evidence of spontaneous recovery (re-emergence of CRs to the CS+), 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 stimulus that previously signalled threat (Bouton, 2002; Phan & Sekhar in Armony & Vuilleumier, 2013; Postle, 2020). This new association of safety therefore competes for expression with the initially acquired threat association. Successful extinction learning, i.e. extinction retrieval, is therefore indexed behaviourally and physiologically by indiscriminate CRs to the CS+ and CS-, whereas continued differential CRs to CS+ are indicative of spontaneous recovery of the initially acquired fear association, i.e. fear retrieval (Levy & Schiller, 2021). Extinction and fear retrieval therefore respectively represent the successful and unsuccessful recall of a change in contingencies indicating that a cue which once signalled threat no longer does.

Findings on the effects of IU in threat acquisition are largely inconclusive. Though research has demonstrated effects of IU on psychophysiological markers of conditioning, where high IU individuals demonstrate heighted and indiscriminate SCRs in response to CS+ vs. CS- compared to

low IU individuals (Chin et al., 2016; Kanen et al., 2021; Morriss, Macdonald, et al., 2016), other studies have failed to find such effects (Mertens & Morriss, 2021; Morriss, 2019; Wake et al., 2021). In their review of the role of IU in conditioning, Morriss et al. (2021) highlighted that varying probabilistic structures of cues during acquisition, notably reinforcement rates, may be at the root of such mixed findings. The researchers suggested that the use of partial, as opposed to continuous, reinforcement may be more likely to demonstrate IU effects on psychophysiological responses during acquisition, as this is more likely to increase perceptions of uncertainty (Morriss et al., 2021).

Research on the effects of IU in extinction demonstrates much clearer patterns. This is likely due to the inherent uncertainty of the extinction phase, as sudden changes in contingencies from threatening to safe are not obvious (Levy & Schiller, 2021). It is this that is thought to maintain CRs in individuals with high levels of IU (Morriss and van Reekum, 2019; Morriss et al., 2019). In addition, as time throughout extinction goes on, levels of uncertainty are also likely to change. This may further depend on the reinforcement rates utilised throughout acquisition, with partial reinforcement rates particularly perpetuating perceptions of uncertainty throughout extinction (Bouton, 2002; Morriss et al., 2021). It is consequently important to consider extinction in terms of two temporal periods (early and late), as opposed to analysing psychophysiological responses as a single phase. This ascertains whether the inherent shifts in uncertainty of the paradigm affect the adjustment of contingencies, and consequently the observed CRs.

Indeed, in early extinction (the first 8 trials), individuals low in IU typically show greater physiological responses to cues that previously signalled threat (CS+), compared with those that signal safety (CS-) (Dunsmoor et al.,2015; Morriss,2019; Tanovic et al.,2018). In late extinction (last 8 trials), low IU individuals demonstrate comparable responses to both CSs, reflecting successful extinction retrieval. Contrastingly, higher levels of self-reported IU have consistently been related to disrupted extinction learning: such individuals typically show elevated CRs to both CSs during early extinction, suggesting threat generalisation. It is during late extinction that high IU individuals begin to demonstrate the same response that low individuals show in early extinction, i.e. high IU individuals demonstrate greater differentiated CRs to the CS+ relative to the CS-, as indexed by greater SCRs, corrugator supercilii activity, and pupil dilation (Morriss, 2019; Morriss, Christakou, et al., 2016; Morriss et al., 2015; Morriss & van Reekum, 2019). Therefore, CRs and the subsequent success of extinction retrieval are engaged in a differential manner as a function of temporal patterns in extinction and individual differences in levels of IU. However, though a vast number of studies reported this pattern of results (Morriss, 2019; Morriss et al., 2015; Morriss et al., 2016a; Morriss et al., 2019; Morriss and van Reekum, 2019), these findings are not always replicated (Morriss et al., 2020).

Furthermore, despite advancements in understanding the involvement of IU in conditioning paradigms, many factors, including sex, genetic polymorphisms, personality traits, brain morphology, and stress, can influence threat-conditioned skin conductance responses (which are the predominant

index of IU in conditioning in the literature), making these highly variable across individuals (Dawson et al., 2007). In addition, the slower temporal resolution of SCRs can result in convolved responses, particularly in designs with short inter-stimulus intervals (Ojala & Bach, 2020), and SCRs can be generated by numerous internal or external processes, that are not specific to particular cognitive processes (Dawson et al., 2007b). Overall, such limitations encourage exploration of other indices of CRs, and the use of eye-tracking may prove particularly beneficial in offering clarification and unique explanations of how distinct forms of IU modulate threat acquisition and extinction.

1.3 Eye-Tracking and Conditioning Paradigms

Eye-movements are thought to provide a high-resolution and overt index of attentional deployment (Rayner, 2009). Due to the anatomy of the eye, high-acuity vision is limited to a small portion of the visual field - the fovea (Holqvsist & Andersson, 2017). As a result, individuals have a strong tendency to move their eyes so that the fovea is directed at stimuli that are being thought of or processed in the moment, which makes eye-tracking a reliable method for observing the allocation of visual attention, as well as attentional avoidance (Carter & Luke, 2020; Felmingham et al., 2011). Global measures of eye-movements are indexed specifically by the number of discrete pauses of the eyes on a stimulus, the length of time in which the eye pauses on a stimulus, and the angular distance traversed between successive fixations, which are termed the number of fixations, fixation duration, and saccade amplitude, respectively (Holmqvist & Andersson, 2017; Hannula et al., 2010). Attentional bias, or hyper-vigilance, is thought to be indexed by global eye-movements such as fewer but longer fixations with decreased saccade amplitude, whereas hyper-scanning, a pattern of behaviour which is thought to represent avoidance through a series of brief glances, is indexed by a higher number of shorter fixations and increased saccade amplitude (Horley et al., 2003).

As attending to threat signals is adaptively essential for animals and humans, using gaze to gather information about possible threats has potential benefits in threat acquisition and extinction. However, literature on the use of eye-tracking measures in conditioning literature is scarce, and existing findings are generally inconclusive. Certain studies report differentiation between threat and safety cues, indexed by longer fixation duration to CS+ relative to CS- (Austin and Duka, 2010; Koenig et al., 2017), whilst others have failed to find such differences (Eippert et al., 2012), or have found contradicting evidence demonstrating longer fixation duration for threat as well as safety cues (Hopkins et al., 2015). Recently, Xia et al. (2020) explored the effects of conditioning using global measures of eye-tracking. The researchers found CRs as measured by eye-tracking, indexed by longer fixation duration and decreased saccade amplitude to CS+ relative to CS- during acquisition, and an absence of differentiation between the cues during extinction. However, Xia et al. did not examine whether effects differed in early vs. late extinction, and did not explore any individual differences in conditioning. The proposed research aims to expand these findings by doings so, and by additionally introducing IU into research on eye-tracking and conditioning.

1.4 Eye-Tracking and Intolerance of Uncertainty

A large body of research has demonstrated that animals as well as humans exhibit sustained vigilance when faced with uncertainty, particularly in states of threat uncertainty, where increases in attentional bias may reflect information-gathering behaviour (Davies & Craske, 2015; Dietrich, Endrass, & Kathmann, 2016; Grupe & Nitschke, 2011; Herry et al., 2007; Hogarth, Dickinson, Austin, Brown & Duka, 2008; Lin et al., 2015; Ran, Chen, Zhang, Ma & Zhang, 2016; Whalen, 2007). From this, it can be inferred that individuals who are highly intolerant of uncertainty are likely to be under increased states of vigilance in uncertain situations and display more information-gathering behaviours, such as fewer but longer fixations with decreased saccade amplitude, compared with low IU individuals. Consequently, individuals high in IU may therefore demonstrate hyper-vigilance to uncertain threat stimuli, such as a partially reinforced CS during acquisition, and in the absence of a CS-US pairing in early extinction, where contingencies suddenly change, and continue to do so throughout late extinction.

However, as described above, when faced with uncertainty, individuals with high levels of IU adopt approach or avoidance behaviours, which are characterised by engagement in information-gathering behaviours to reduce uncertainty, and in avoidance-based strategies to reduce uncertainty exposure, respectively (Birrell et al., 2021). Furthermore, as IU is thought to comprise of P-IU and I-IU dimensions, which are related to opposing psychophysiological responses (Jackson et al., 2016; Nelson et al., 2015), it is therefore possible that high levels of IU may modulate either hyper-vigilance or hyper-scanning behaviours. High IU individuals may demonstrate excessive search for threat cues, reflecting a state of hyper-vigilance or attentional bias, or may engage in avoidance and hyper-scanning behaviours. The use of eye-tracking methodology may therefore prove particularly beneficial in differentiating such responses and delineating the nature of attentional bias in IU.

1.5 The Present Study

This project involved a secondary analysis of a previously published dataset (Morriss et al., 2020), in which expectancy ratings, eye-tracking, pupil dilation, and SCRs were recorded in a conditioning paradigm with threat acquisition, same-day extinction, and next-day extinction phases. Please note that the focus of this project was on the threat acquisition and same-day extinction eye-tracking data, which was not reported in the publication.

The proposed analysis aimed to (1) replicate existing but separate findings on eye-tracking in conditioning (Koenig et al., 2017; Xia et al., 2020) and on the effects of IU in conditioning paradigms (Morriss, 2019; Morriss, Christakou, et al., 2016; Morriss et al., 2015; Morriss & van Reekum, 2019), and (2) to extend these findings and add to the literature by examining whether the methodology of eye-tracking could be applied in the context of IU. Based on the evidence evaluated above, it was

hypothesised that, during acquisition, CRs would be observed, indexed by (1) fewer fixations, (2) longer fixation duration, and (3) decreased saccade amplitude to the threat (CS+) relative to the safety (CS-) cues. Due to the partial reinforcement rate utilised in this study, we expected to find some effects of IU during acquisition, with high IU individuals demonstrating stronger attentional bias to both threat and safety cues compared with low IU individuals (even fewer fixations, even longer fixation duration, and more decreased saccade amplitude than low IU individuals).

Furthermore, based on previous research on eye-tracking in conditioning (Koenig et al., 2017; Xia et al., 2020), as well as existing research on IU in extinction (Morriss, 2019; Morriss et al., 2015; Morriss and van Reekum, 2019), it was predicted that during early extinction (first 8 trials), both high and low IU individuals would demonstrate attentional bias towards the CS+ vs. CS-, as indexed by (1) fewer fixations, (2) longer fixation duration, and (3) decreased saccade amplitude. However, it was further predicted that, during late extinction (last 8 trials), differentiated responses to the CS+ vs. CS- would extinguish in low IU individuals, with no differences in responses to CS+ vs CS-, but that high IU individuals would continue to demonstrate CRs the threat vs. the safety cues, as indexed by (1) fewer fixations, (2) longer fixation duration, and (3) decreased saccade amplitude to the CS+ relative to CS-.

Finally, an additional aim was to further our understanding of the contribution of IU versus trait anxiety to conditioned responses. The latter was therefore controlled for, as in line with previous work investigating specificity of IU in threat acquisition and extinction (see Morriss, Christakou, et al., 2016).

2. METHOD

2.1 Participants

For the original study (Morriss et al., 2020), a sample of 144 participants was recruited from the University of Reading local area using advertisements and word of mouth. Participants between 18 and 35 years of age were recruited due to differences in conditioning resulting from age and hormone levels in populations under or over this range (Lonsdorf & Merz, 2017). A total of five participants were excluded due to poor data quality and optic artefacts (multiple trials with missing data/ failure to track pupil), resulting in a final sample of n = 139 (Age: M = 24.14, SD = 4.42, range = 18-35; Ethnicity: 87 White, 29 Asian, 4 Middle Eastern/Arab, 2 Black, 2 Mixed, and 15 not specified; Sex: 83 female, 54 male, 2 not specified; Sexual Orientation: 103 Heterosexual, 18 Sexual Minorities (lesbian / gay/ bisexual / pansexual), 18 not specified). Please refer to Appendix A for detailed participant exclusion protocol. To verify that findings were unlikely to be confounded due to demographic differences between groups, high and low IU groups were tested for significant differences in terms of age, ethnicity, sex, and sexual orientation. Please see Appendix B for results of these analyses.

For the proposed analyses, an a-priori sample size calculation to detect the main effects of IU, Stimulus, and their interaction was estimated using G*Power (Faul et al., 2009) using a repeated-measures within-between interaction design. The following parameters were entered: f = .26, α = .05, β = .95, number of groups = 2 (IU: high/low), number of measurements = 4 (max. per phase, e.g. Extinction: Early/Late & CS+/CS-). The total sample size suggested was n = 34. 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 on eye-tracking with stimulus timings which most closely match the design of the current study (3s time-period before US onset). The power parameter of .95 was chosen to decrease the probability of committing a Type II error, particularly as, to our knowledge, this is the first study to investigate eye-tracking within the context of IU and conditioning together.

Furthermore, as analyses of individual differences tend to have associated 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, and all other parameters as reported above. The total sample size suggested was n = 130. Therefore, though we overpowered our main effects analyses, our sample size was appropriate for analyses of individual differences in IU.

2.2 Ethical Considerations

Ethical approval for this study was granted by the University of Reading Research Ethics Committee. 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). Participants were made aware of their right to withdraw from the study at any point without having to provide a reason. Participants were provided with an information sheet detailing an overview and the purposes of the study, they granted written informed consent, and were fully debriefed at the end of the study. There is no breach of ethics in conducting this secondary analysis. Personally identifiable information is inaccessible, and data were stored securely, handled with confidentiality, and will be disposed of upon completion of the project.

2.3 Design

A 2 x 2 between-within design was used to test the effects of IU (high, low) and Stimulus (CS+, CS-) in acquisition, and a 2 x 2 x 2 between-within design tested the effects of IU (high, low), Stimulus (CS+, CS-) and Time (early, late) for the extinction phase, with separate analyses conducted on each of the dependent variables (DVs: fixation count, fixation duration, saccade amplitude). Participants were assigned to IU groups based on a median split, depending on whether they scored high (above average, ≤ 65) or low (below average, > 65) on the IU questionnaire (Freeston et al., 1994). As we were interested in temporal patterns throughout the extinction phase, this resulted in a total of two between (High IU and Low IU) and six within conditions: Acquisition CS+, Acquisition CS-, Early Extinction CS+, Early Extinction CS-, Late Extinction CS+, Late Extinction CS-. To test specificity of IU over trait anxiety, total scores on a measure of anxiety were entered into additional analyses as a covariate.

2.4 Overall Procedure

At the start of the session, participants were informed about the experimental procedure and seated at the testing booth, where they provided informed consent and completed questionnaires (see below). Participants were asked to remove eye make-up, as areas of darkness around the eye, such as eye-lashes darkened by make-up, can make the pupil hard to identify (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. The experiment was performed in a dark room and the testing session lasted approximately 30 minutes.

2.5 Apparatus

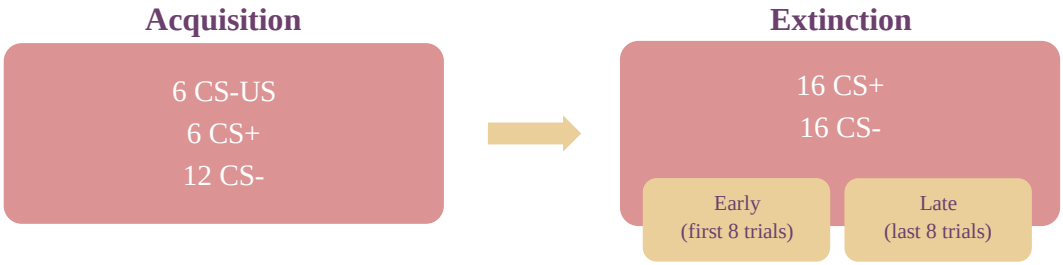
Eye-movements were recorded monocularly (right eye only) using a head-mounted Eyelink II eye-tracker and pupil-only tracking mode with a sampling rate of 250 Hz, spatial resolution (RMS) of < 0.01°, and temporal resolution of 4ms (EyeLink II Manual, SR Research). Head movements were constrained with a chinrest at a viewing distance of 57cm. 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 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 (Sennheiser HD 206).

2.6 Stimuli

The CSs were monochromatic squares (blue: RGB values 205, 236, 255 and yellow: RGB values 255, 255, 3) with 233 x 233 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., 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 prior to each session.

Figure 1

Experimental Conditions and Procedure.



2.7 Conditioning Task

The conditioning task was designed and presented using E-Prime 2.0 software (Psychology Software Tools Ltd., Pittsburgh, PA). The task comprised of two learning phases: threat acquisition and extinction. There were 24 trials in the acquisition phase and 32 trials in the extinction phase (see Fig. 1), with two blocks for each phase. Early extinction was defined as the first 8 CS+/CS- trials, and late extinction as the last 8 CS+/CS- trials. During acquisition, one of the stimuli (blue or yellow square) (CS+) was paired with the aversive sound (CS-US), whilst the other stimulus (yellow or blue

square) was presented alone (CS-). A 50% reinforcement schedule was used to maximise unpredictability of the CS-US contingency (Morriss et al., 2020). During extinction, both CSs were presented without the US.

Participants were not instructed on contingencies or informed about the number of CS's. 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. CSs were always centred on the screen and presented for a total of 4000ms. The US was presented for 1000ms and co-terminated with the reinforced CS+. Following this, a blank black screen was presented for 6000-8800ms.

2.8 Questionnaires

2.8.1 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, $\alpha = .91$ (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 all characteristic of me,* and 5 = *entirely characteristic of me.* Total scores range from 27-135, with higher scores indicating higher levels of IU.

2.8.2 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, $\alpha s > .87$ (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 for range from 21-84, with higher scores indicating higher levels of trait anxiety.

2.9 Data Preparation

2.9.1 Scoring of Questionnaire Data

Questionnaire responses were checked for completeness and scored according to their respective manuals. Please refer to Appendices C and D for full scales and detailed scoring instructions. Data were checked for extreme scores through visual inspection of frequency distributions and computations of the range. All entries were within the range of possible scores and

were therefore accepted as valid responses. Checks for outliers were not performed on IUS and STICSA scores, as any identified outliers may reflect true variation in levels of IU or trait anxiety. There were therefore no exclusions identified at this point.

2.9.2 Pre-Processing of Eye-Tracking Data

Raw eye-tracking data were automatically segmented online into sequences of saccades and fixations using the EyeLink II parsing system and standard cognitive configuration to identify the start and ends of saccades, with 30°/s velocity, and 8000°/s^2 acceleration criteria (SR Research, Manual). Eye-movements that did not meet these criteria were pre-defined as fixations, as is common in eye-tracking literature (Holmqvist & Andersson, 2017). Following this, data were visually inspected for quality offline using DataViewer (version 4.2.1), at which point participant exclusions were identified, and fixation and saccade reports with variables of interest were generated. As overall attention reflects the viewing pattern 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 Xia et al. (2020), were quantified:

(1) fixation count (number of discrete pauses of the eyes on the CS)

(2) fixation duration (length of time for which the eye pauses on the CS (ms))

(3) saccade amplitude (angular distance traversed between successive fixations (°/s)).

Finally, the data were cleaned using R (version 4.0.2), as follows: CS-US trials were discarded to avoid sound confounds. Eye-movements that began prior to stimulus onset or following stimulus offset were excluded from analyses, as partial events such as these could result in artificially low fixation durations (Holmqvist & Andersson, 2017), and we were interested in eye-movements in response to the stimuli specifically. In order to obtain a complete overview of global attention, no further exclusion criteria for saccades or fixations were adopted, and all movements were accepted as legitimate responses to stimuli. Trials were averaged for 4000ms following CS onset per stimulus type and conditioning phase for each participant, resulting in the following conditions for each of the quantified variables: Acquisition CS+, Acquisition CS-, Extinction CS+, Extinction CS-, Early Extinction CS+, Early Extinction CS-, Late Extinction CS+, Late Extinction CS-. Please refer to Appendices E and F for detailed data pre-processing protocol and scripted data cleaning, respectively.

Oculomotor measures are idiosyncratic and their distributions are typically positively skewed (Holmqvist & Andersson, 2017). Data were therefore visually inspected for non-normal distributions and evaluated for skew values ± 3. As the data for Early Extinction CS- and Late Extinction CS- fixation duration had skew values > 3 in the high IU group (3.41 and 3.05, respectively), fixation duration was log-transformed for all conditions. Please see Appendix G for frequency distributions of these variables pre- and post-log-transformation.

3. RESULTS

Statistical analyses were conducted in R version 4.0.2. Please see Appendix H for fully scripted analyses and Appendix I for assumption tests. An alpha level of .05 was used throughout to denote significance. Effect sizes were interpreted based on Cohen's (1988) conventions.

3.1 Questionnaires

The reliability of IUS and STICSA questionnaires was satisfactory, with respective internal consistencies of $\alpha = .95$ and $\alpha = .88$. Data for each measure approximated normal distributions in both IU groups (see Fig. 2). IUS scores ranged from 65-125 in the high IU group ($M = 82.65$, $SD = 14.77$), and from 32-64 in the low IU group ($M = 49.70$, $SD = 8.51$). STICSA scores ranged from 30-69 in the high IU group ($M = 45.29$, $SD = 9.30$) and from 22-57 in the low IU group ($M = 35.99$, $SD = 7.32$).

Figure 2

Frequency Distributions of (a.) IUS-27 and (b.) STICSA Measures by IU Group.

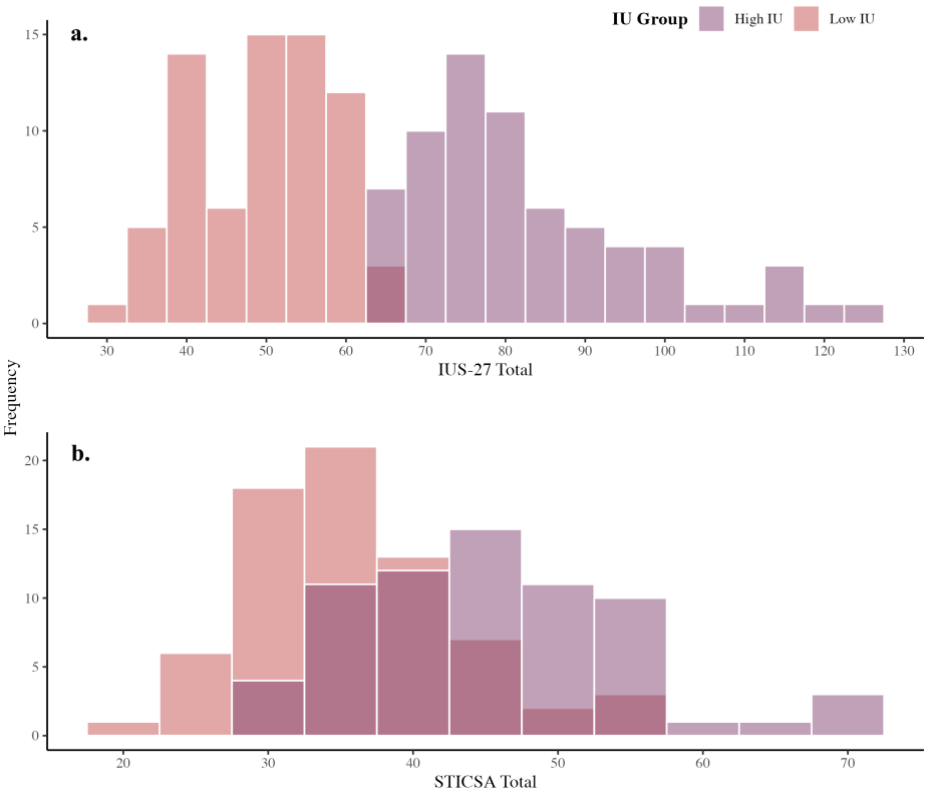


Table 1

Summary of Descriptive Statistics for Eye-Tracking Measures as a Function of Conditioning Phase and Stimulus Type.

	Acquisition				Early Extinction				Late Extinction			
	CS+		CS-		CS+		CS-		CS+		CS-	
	n	*M (SD)*	*n*	*M (SD)*	*n*	*M (SD)*	*n*	*M (SD)*	*n*	*M (SD)*	*n*	*M (SD)*
High IU												
Fixation Count	68	7.51 (3.84)	68	7.97 (3.07)	68	7.54 (3.26)	68	8.14 (3.26)	68	8.41 (3.63)	68	8.89 (3.33)
Fixation Duration (ms) [a]	68	6.68 (0.87)	68	6.60 (0.78)	68	6.54 (0.70)	68	6.40 (0.75)	68	6.41 (0.72)	68	6.31 (0.71)
Saccade Amplitude (°/ms)	68	3.10 (1.71)	67	3.16 (1.70)	68	3.21 (1.80)	67	3.46 (1.88)	68	3.21 (1.78)	68	3.37 (1.85)
Low IU												
Fixation Count	71	6.33 (3.38)	71	6.67 (3.31)	71	6.80 (4.06)	71	6.70 (3.66)	71	6.72 (3.15)	71	6.87 (3.43)
Fixation Duration (ms) [a]	71	6.92 (0.89)	71	6.91 (0.85)	71	6.81 (0.94)	71	6.92 (0.96)	71	6.79 (0.89)	71	6.81 (0.91)
Saccade Amplitude (°/ms)	71	2.66 (1.27)	70	2.80 (1.43)	70	2.95 (1.83)	71	2.81 (1.53)	70	2.79 (2.03)	71	2.84 (2.06)

Note. Ms = milliseconds; °/ms = degrees per millisecond.

[a] Fixation duration data reported here are the geometric (log-transformed) mean. For arithmetic mean, please refer to Appendix J.

3.2 Effects of IU, Stimulus, and Their Interaction on Eye-Tracking

See Table 1 for descriptive statistics of eye-movement variables. For the acquisition phase, to test the effects of IU, Stimulus, and their combined influence on eye-movements, a total of three 2 x 2 mixed ANOVAs were computed on each of the three eye-tracking measures (fixation count, fixation duration, and saccade amplitude). IU (high, low) and Stimulus (CS+, CS-) were entered as the between and within factors, respectively. For the extinction phase, to test the effects of IU, Stimulus, Time and their interaction in extinction, a total of three 2 x 2 x 2 mixed ANOVAs were computed on the eye-tracking measures (fixation count, fixation duration, and saccade amplitude), with IU (high, low) entered as the between, and Stimulus (CS+, CS-) and Time (early, late) entered as the within factors. In the case of significant interactions and in line with a-priori hypotheses, simple main effects were then conducted to better understand the patterns of results.

3.2.1 Acquisition

The ANOVA revealed a statistically significant main effect of IU [$F(1, 137) = 4.81$, $p = .030$, $\eta^2_p = .034$] and Stimulus [$F(1,137) = 11.44$, $p < .001$, $\eta^2_p = .077$], but a non-significant IU-Stimulus interaction [$F(1,137) = 0.26$, $p = .613$, $\eta^2_p = .002$] on fixation count. As demonstrated by the descriptive statistics in Table 1 and in Figure 3, the high IU group had higher fixation counts in response to both stimuli than the low IU group, and there were fewer fixations in response to the CS+ versus the CS-.

Furthermore, the ANOVA demonstrated a significant main effect of IU [$F(1,137) = 3.91$, $p = .050$, $\eta^2_p = .028$] on fixation duration, with shorter fixation durations in the high IU group (CS+: $M = 6.68$; CS-: $M = 6.60$) than in the low IU group (CS+: $M = 6.92$; CS-: $M = 6.91$). There was no significant effect of Stimulus [$F(1,137) = 2.92$, $p = .090$, $\eta^2_p = .021$] or IU-Stimulus interaction [$F(1,137) = 1.27$, $p = .261$, $\eta^2_p = .000$].

There were no significant main effects of IU [$F(1,136) = 2.98$, $p = .086$, $\eta^2_p = .022$] , Stimulus [$F(1,136) = 0.95$, $p = .332$, $\eta^2_p = .007$], and no significant IU-Stimulus interaction [$F(1,136) = 0.38$, $p = .539$, $\eta^2_p = .003$] on saccade amplitude throughout acquisition.

3.2.2 Extinction

The ANOVA revealed a significant main effect of IU [$F(1,137) = 7.67$, $p = .006$, $\eta^2_p = .053$], Stimulus [$F(1,137) = 4.16$, $p = .043$, $\eta^2_p = .029$], and Time [$F(1,137) = 5.73$, $p = .018$, $\eta^2_p = .049$] on fixation count. As reported in Table 1, high IU individuals therefore had significantly fewer fixations than low IU individuals. There were also significantly fewer fixations to the CS+ than the CS-, and fewer fixations in early versus late extinction. Furthermore, there was a statistically significant interaction between IU and Time [$F(1,137) = 4.57$, $p = .034$, $\eta^2_p = .032$] on fixation count, and non-statistically significant interactions between IU-Stimulus [$F(1,137) = 3.46$, $p = .065$, $\eta^2_p = .025$],

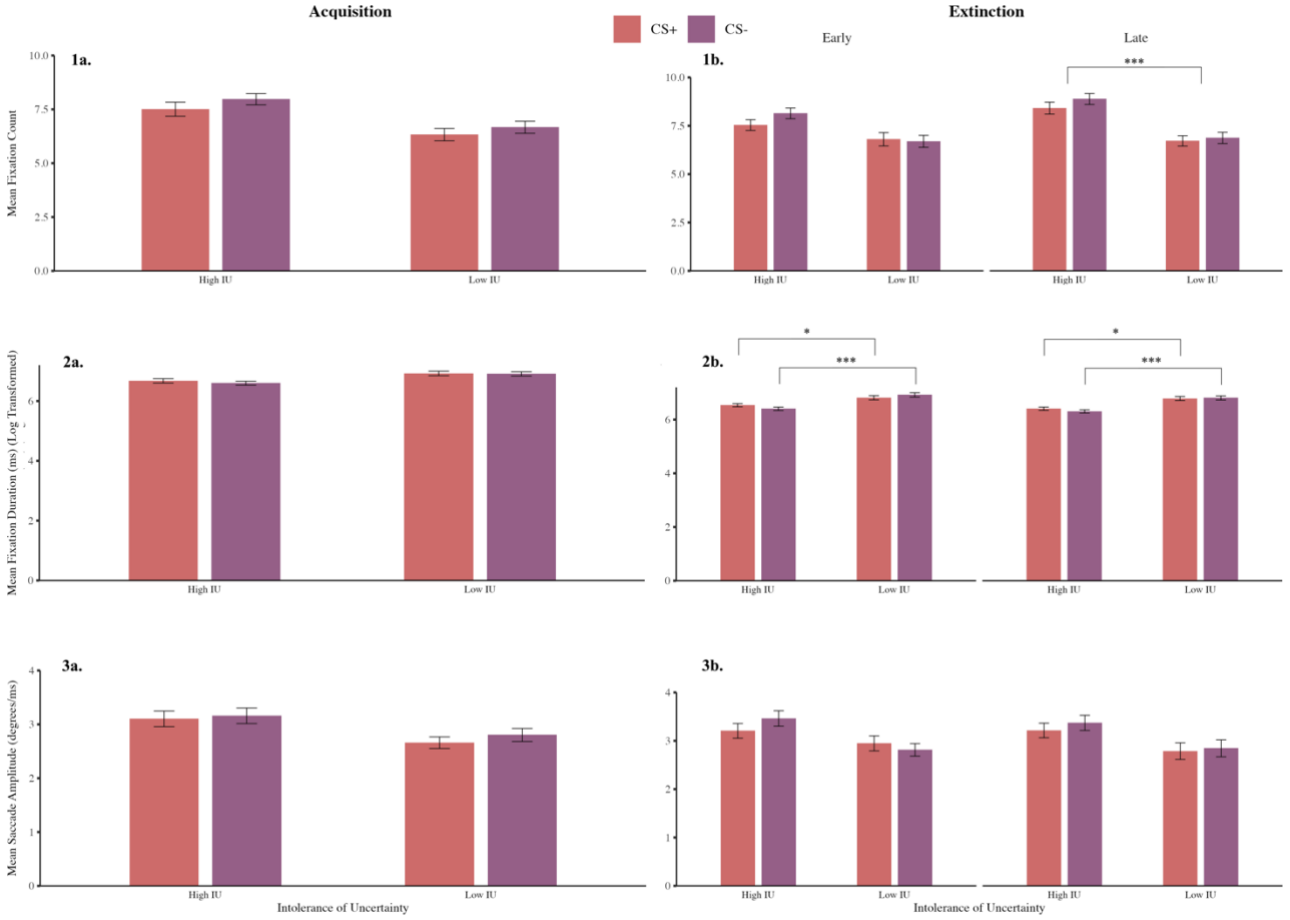
Stimulus-Time [$F(1,137) = 0.06$, $p = .806$, $\eta^2_p < .001$], or IU-Stimulus-Time [$F(1,137) = 0.60$, $p = .440$, $\eta^2_p = .004$]. Simple main effects revealed that there was a statistically significant difference in fixation count between IU groups specifically during late extinction [$F(1,137) = 11.41$, $p < .001$, $\eta^2_p = .077$], whereby high IU individuals demonstrated a higher number of fixations (CS+: $M = 8.41$; CS-: $M = 8.89$) than low IU individuals (CS+: $M = 6.72$; CS-: $M = 6.81$). Though there was a similar pattern in early extinction, this was not statistically significant [$F(1,137) = 3.63$, $p = .059$, $\eta^2_p = .011$]. The interaction therefore appeared to be driven by differential responses between IU groups in late extinction only.

Furthermore, there was a statistically significant main effect of IU [$F(1,137) = 11.21$, $p < .001$, $\eta^2_p = .076$], Time [$F(1,137) = 4.35$, $p = .039$, $\eta^2_p = .031$], and a significant IU-Stimulus interaction [$F(1,137) = 5.82$, $p = .018$, $\eta^2_p = .041$] on fixation duration in extinction. There were no further significant main effects of Stimulus [$F(1, 137) = 0.51$, $p = .477$, $\eta^2_p = .004$], or interaction effects on fixation duration throughout extinction: IU-Time [$F(1,137) = 0.24$, $p = .624$, $\eta^2_p = .002$], Stimulus-Time [$F(1,137) = 0.16$, $p = .680$, $\eta^2_p = .001$], IU-Stimulus-Time [$F(1,137) = 0.95$, $p = .333$, $\eta^2_p = .007$]). As can be seen in Table 1 and Figure 3, the high IU group demonstrated shorter fixation durations than the low IU group, and fixation durations were overall longer in early versus late extinction. Simple main effects revealed that differences in fixation duration between IU groups in response to the CS+ were statistically significant [$F(1,137) = 6.70$, $p = .011$, $\eta^2_p = .047$], with high IU individuals demonstrating shorter fixation durations throughout extinction (Early: $M = 6.54$, Late: $M = 6.41$) than low IU individuals (Early: $M = 6.81$, Late: $M = 6.79$). This pattern was similar in response to the CS- as high IU individuals also demonstrated shorter fixation durations (Early: $M = 6.40$, Late: $M = 6.31$) than low IU individuals (Early: $M = 6.92$, Late: $M = 6.81$), and this was statistically significant [$F(1,137) = 14.43$, $p < .001$, $\eta^2_p = .095$].

There were no significant main effects or interactions throughout extinction on saccade amplitude: IU [$F(1,134) = 3.17$, $p = .077$, $\eta^2_p = .023$], Stimulus [$F(1,134) = 0.74$, $p = .391$, $\eta^2_p = .005$], Time: [$F(1,134) = 0.28$, $p = .601$, $\eta^2_p = .002$], IU-Stimulus [$F(1, 134) = 1.69$, $p = .196$, $\eta^2_p = .012$], IU-Time [$F(1,134) = 0.13$, $p = .718$, $\eta^2_p < .001$], Stimulus-Time [$F(1,134) = 0.08$, $p = .781$, $\eta^2_p < .001$], IU-Stimulus-Time [$F(1,134) = .61$, $p = .437$, $\eta^2_p < .001$].

Figure 3

Bar Graphs Depicting Mean (1a & 1b) Number of Fixations, (2a & 2b) Fixation Duration, and (2a & 2b) Saccade Amplitude for Each IU Group and Stimulus Type by Acquisition and Extinction Phase.



Note. Error bars denote standard error of the mean.

* $p < .05$, ** $p < .01$, *** $p < .001$.

3.3 Specificity of IU over Trait Anxiety

To further assess whether the observed effects were specific to IU, or whether they could be explained by individual differences in levels of trait anxiety, a series of ANCOVAs were carried out. The above mixed ANOVA analyses were therefore repeated with mean-centred STICSA totals entered as a covariate. In the case of significant main effects or interactions, estimated marginal means were computed and are reported below. Furthermore, as above, in the case of significant interactions and in line with a-priori hypotheses, simple main effects were then conducted to better understand the patterns of results.

3.3.1 Acquisition

The ANCOVA revealed a significant main effect of Stimulus [$F(1,136) = 11.62$, $p < .001$] on fixation count in acquisition, which was associated with a medium effect size, $\eta^2_p = .079$, and demonstrated a pattern of lower fixation count to the CS+ ($M = 6.92$) relative to CS- ($M = 7.32$). Despite a non-significant effect of STICSA [$F(1,136) = 0.06$, $p = .808$, $\eta^2_p = < .001$], the analyses further revealed that the main effect of IU was no longer significant after controlling for STICSA [$F(1,136) = 3.19$, $p = .076$, $\eta^2_p = .023$]. This demonstrates that there is no specificity of IU over trait anxiety on fixation count in acquisition. The IU-Stimulus interaction remained non-significant after controlling for STICSA [$F(1,136) = 1.23$, $p = .269$, $\eta^2_p = .009$].

Furthermore, there were no significant main effects or interactions on fixation duration throughout acquisition after controlling for trait anxiety: IU [$F(1,136) = 3.89$, $p = .051$, $\eta^2_p = .028$], Stimulus [$F(1,136) = 2.94$, $p = .089$, $\eta^2_p = .021$], STICSA [$F(1,136) = 0.27$, $p = .606$, $\eta^2_p = .002$], IU-Stimulus [$F(1,136) = 1.67$, $p = .198$, $\eta^2_p = .012$]. As the effect of IU was no longer significant after controlling for STICSA, this demonstrates that the previously observed effect of IU on fixation duration in acquisition was not specific to IU.

The ANCOVA further revealed there were no significant main effects of IU [$F(1,134) = 2,13$, $p = .147$, $\eta^2_p = .016$], Stimulus [$F(1,134) = 0.94$, $p = .333$, $\eta^2_p = .007$] and STICSA [$F(1,134) = 0.01$, $p = .935$, $\eta^2_p < .001$] on saccade amplitude. The IU-Stimulus interaction was likewise non-significant [$F(1,134) = 0.86$, $p = .354$, $\eta^2_p = .006$].

3.3.2 Extinction

ANCOVA analyses revealed a significant main effect of IU [$F(1,136) = 4.36$, $p = .039$, $\eta^2_p = .031$], Stimulus [$F(1,136) = 4.21$, $p = .042$, $\eta^2_p = .030$], and Time [$F(1,136) = 5.69$, $p = .018$, $\eta^2_p = .040$] on fixation count in extinction. There were more fixations for high IU individuals ($M = 8.15$) than low IU individuals ($M = 6.87$), and more fixations in response to the CS- ($M = 7.65$) than CS+ ($M = 7.37$). There were fewer fixations throughout early extinction ($M = 7.29$) when compared with late extinction ($M = 7.72$). There was no significant main effect of STICSA [$F(1,136) = 0.43$, $p =$

.512, η^2_p = .003]. The previously observed IU-Time interaction was no longer significant after controlling for STICSA [F(1,136) = 3.49, p = .064, η^2_p = .025], which demonstrates that this interaction was not specific to IU but could instead be attributed to individual differences in trait anxiety. Furthermore, after controlling for STICSA, there was a significant IU-Stimulus interaction [F(1,136) = 4.56, p = .035, η^2_p = .032]. Simple main effects revealed that there was a statistically significant difference in fixation count between IU groups specifically in response to the CS- [F(1,136) = 6.66, p = .011, η^2_p = .047], whereby high IU individuals demonstrated a higher number of fixations (M = 8.46) than low IU individuals (M = 6.84). Though this pattern was similar in response to the CS+ (High IU: M = 7.84; Low IU: M = 6.90), this was not statistically significant [F(1,136) = 2.17, p = .143, η^2_p = .016]. The interaction therefore appeared to be driven by differential responses between IU groups towards the CS- only. The IU-Stimulus-Time interaction was not significant [F(1,136) = 0.04, p = .834, η^2_p < .001].

In addition, there continued to be a significant main effect of IU [F(1,136) = 8.37, p = .004, η^2_p = .058] on fixation duration in extinction after controlling for STICSA, which suggests that this effect is specific to IU over trait anxiety. In addition, the observed main effect of Time continued to be significant after accounting for trait anxiety [F(1,136) = 4.36, p = .039, η^2_p = .031]. The pattern remained the same, with high IU individuals demonstrating shorter fixations than low IU individuals, and overall shorter fixations throughout early versus late extinction, as seen in Figure 3. The observed IU-Stimulus interaction likewise remained significant after controlling for STICSA, [F(1,136) = 5.36, p = .022, η^2_p = .038]. Similarly to the simple main effects following the observed mixed ANOVA, simple main effects for the ANCOVA revealed that differences in fixation duration between IU groups in response to both stimuli were statistically significant (CS+[F(1,136) = 4.70, p = .032, η^2_p = .033], CS- [F(1,136) = 11.19, p = .001, η^2_p = .076]. There was no significant main effect of STICSA [F(1,136) = 0.01, p = .972, η^2_p < .001], and the effect of Stimulus [F(1,136) = 0.51, p = .475, η^2_p = .004] remained non-significant even after controlling for trait anxiety. All remaining interactions likewise remained non-significant in ANCOVA analyses: IU-Time [F(1,136) = 0.50, p = .480, η^2_p = .004], Stimulus-Time [F(1,136) = 0.17, p = .677, η^2_p = .001], IU-Stimulus-Time [F(1,136) = 0.34, .539, η^2_p = .003].

The ANCOVA analyses further demonstrated a similar pattern throughout extinction on saccade amplitude as the observed mixed ANOVA effects, with no significant main effects or interactions even after controlling for trait anxiety: STICSA [F(1,133) = 1.13, p = .289, η^2_p = .008], IU [F(1,133) = 1.03, p = .313, η^2_p = .008], Stimulus [F(1,133) = 0.75, p = .387, η^2_p = .006], Time: [F(1,133) = 0.26, p = .615, η^2_p = .002], IU-Stimulus [F (1,133) = 2.04, p = .156, η^2_p = .015], IU-Time [F (1,133) = 0.80, p = .372, η^2_p = .006], Stimulus-Time [F (1,133) = 0.07, p = .790, η^2_p = .001], IU-Stimulus-Time [F (1,133) = 0.10, p = .32, η^2_p = .007].

4. DISCUSSION

4.1 Summary of Findings

- Summary and general statement about findings
- Were hypotheses supported?
- What can be inferred?

4.2 Application of Findings to Theory and Literature (reverse order from literature review – from specific to more broad)

- To eye-tracking and IU research
- To eye-tracking and conditioning research
- To IU/ anxiety/ conditioning research

4.3 Strengths and Limitations

- Theoretical
    - Inclusion of and accounting for trait anxiety
    - The nature of link between fixation and attention (does fixating on an object actually mean it is being attended?)
- Methodological
    - Drift in the data (no fixation point)
    - Large sample (well powered)
    - Other potential confounds: glasses, lenses, ocular dominance.

4.4 Future Directions

- Replication (especially considering the drift)
- Further analysis accounting for additional effects of sex or violations of parametric assumptions
- Refine eye-tracking measures (more calibration points, more specific definitions of fixations, include fixation point, AOI)
- Introduce IU subscales
- Contribute further to literature by looking at extinction retention (Day 2)

5. CONCLUSIONS

- Closing remarks
    - re-iterate project aims, hypotheses/RQ, methods, broad findings & implications
    - closing statement.

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APPENDICES

Appendix A. Participant Exclusion Protocol

Please refer to document on **https://github.com/crodsob/empirical_project/tree/main/appendices** titled ‘Appendix A –Participant Exclusion Protocol’.

Appendix B. *Tests for Significant Differences in Demographics Variables Between IU Groups*

Please refer to document on **https://github.com/crodsob/empirical_project/tree/main/appendices** titled ‘Appendix B – Tests for Significant Differences in Demographics Variables Between IU Groups’.

Appendix C. *Intolerance of Uncertainty Scale: Scale and Scoring Instructions.*

Please refer to document on **https://github.com/crodsob/empirical_project/tree/main/appendices** titled ‘Appendix C – Intolerance of Uncertainty Scale: Scale and Scoring Instructions’.

Appendix D. *State-Trait Inventory for Cognitive and Somatic Anxiety- Trait Version: Scale and Scoring Instructions*

Please refer to document on **https://github.com/crodsob/empirical_project/tree/main/appendices** titled ‘Appendix D – State-Trait Inventory for Cognitive and Somatic Anxiety- Trait Version: Scale and Scoring Instructions’.

Appendix E. *Data Pre-Processing Protocol*

Please refer to document on **https://github.com/crodsob/empirical_project/tree/main/appendices** titled ‘Appendix E – Data Pre-Processing Protocol’.

Appendix F. *Data Cleaning Script*

Please refer to **https://github.com/crodsob/empirical_project/tree/main/appendices** for full data cleaning script titled ‘Appendix F – Data Cleaning Script’. It is recommended to use the .html version.

Appendix G. *Frequency Distributions of Fixation Duration Variables Pre- and Post-Log-Transformation*

Please refer to document on **https://github.com/crodsob/empirical_project/tree/main/appendices** titled ‘Appendix G – Frequency Distributions of Fixation Duration Variables Pre- and Post-Log-Transformation’.

Appendix H. *Data Analysis Script*

Please refer to **https://github.com/crodsob/empirical_project/tree/main/appendices** for full analysis script titled 'Appendix H – Data Analysis Script'. It is recommended to use the .html version.

Appendix I. Tests for Assumptions of Parametric Analyses

Mixed ANOVA

- Categorical IVs, interval/ratio DVs
- Residuals approximate normal distribution (for each level of each IV)
- homogeneity of variances (at each level of each IV)
- Homoscedasticity (plot standardised residuals against predicted values)
- Sphericity (not applicable in this case, as no within-subjects factors with > 3 levels)
- Homogeneity of variance-covariance matrices

ANCOVA

- Independence of covariate and IVs
- Homogeneity of regression slopes
- Linearity between covariate and DVs at each level of IV grouping (check by creating grouped scatterplot)
- No significant outliers

- Results to be added.

Appendix J. *Arithmetic Mean and Standard Deviation of Fixation Duration as a Function of Phase and Stimulus Type.*

Please refer to document on **https://github.com/crodsob/empirical_project/tree/main/appendices** titled ‘Appendix J – Arithmetic Mean and Standard Deviation of Fixation Duration as a Function of Phase and Stimulus Type’.