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**Dr Carien van Reekum & Dr Jayne Morriss**

Raw data submitted as per instructions □ *Yes*

Consent forms submitted as per instructions □ *n/a*

Word count: 10761

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# School of Psychology and Clinical Language Sciences
# Department of Psychology

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

Claudia Rodriguez-Sobstel

**SEPT 2021**

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Submitted in part fulfilment of the requirement for the degree of
MSc in Cognitive Neuroscience

University of Reading 2020-21

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# ACKNOWLEDGEMENTS

With thanks to my supervisors, Dr Carien van Reekum and Dr Jayne Morriss, for their invaluable support and guidance throughout the course of this project. Your questions and suggestions have helped me gain novel perspectives and challenged me to become a better researcher. Thank you for providing me with an opportunity to continue to pursue a topic of such great interest to me. Eye likewise thank Dr Eugene McSorley for sharing his eye-tracking expertise and teaching me all sorts of tricks in DataViewer.

I would also like to acknowledge Dr Carolyn McNabb and the University of Reading Coding Club. Your mentorship and patience in teaching me the nuances of R inspired a love of coding I never knew was in me. It is thanks to you that I was able to achieve my goal of scripting my dissertation analyses in a reproducible manner.

To those who volunteered their time to participate in the study and to collect the data - the completion of this project would not have been possible without you, and your contributions are sincerely appreciated.

Finally, to my friends and family, for filling my room with plants and for providing unending reassurance, despite my questionable choice of completing two MSc programmes in the midst of a pandemic. I am overwhelmed by the unwavering encouragement I have received from you to follow my passions (and I promise I won't be undertaking another MSc any time soon!).

I am profoundly grateful to you all.

# ABSTRACT

***Background:*** Individuals with high levels of Intolerance of Uncertainty (IU) have difficulty updating threat and safety associations. Recently, eye-tracking methodology has been demonstrated to be a valid psychophysiological measure of conditioned responses in conditioning paradigms. As high IU individuals tend to avoid uncertainty or seek out disproportionate amounts of information in such situations, here we assessed hyper-vigilant and hyper-scanning behaviours by using eye-tracking as a direct index of visual information seeking. This was measured in the context of a conditioning paradigm, with threat acquisition and extinction phases, due to the inherent uncertainty of the extinction phase. ***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 ($n$ = 139) 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. 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. Effects were attributed to individual differences in IU over trait anxiety in extinction, but not in acquisition. ***Conclusions:*** These findings demonstrate that eye-tracking can be added to the toolbox of methodologies utilised to study IU in conditioning paradigms. Our results inform models of IU, trait anxiety, attentional bias, as well as attention- 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 highlighted 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). Intolerance of uncertainty is thought to stem from a fundamental fear of the unknown and has been identified as an important facet of larger constructs such as anxiety and neuroticism (Carleton, 2016a, 2016b; Dugas et al., 2004; Grupe & Nitschke, 2013). Whereas trait anxiety is associated with aversive responding to potential threat (Armstrong & Olatunji, 2012; Bar-Haim et al., 2007; Beck & Clark, 1997; Mathews & MacLeod, 1985; Wermes et al., 2018), high levels of self-reported IU are thought to increase susceptibility to excessive worry at times of uncertainty, as it is the perceived absence of information that is thought to trigger aversive responding in such individuals (Buhr & Dugas, 2002; Carleton, 2016a). Given growing evidence of its ubiquity (Berenbaum et al., 2008; Carleton, 2016a; Grupe & Nitschke, 2013; Norton, 2005) and transdiagnostic role in anxiety and mood 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.

## 1.1. 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, 2013; Harrison et al., 2013; LeDoux, 1998). A 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 (LeDoux, 1998). 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 cue 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, i.e. extinction retrieval, is therefore indexed by indiscriminate CRs to the CS+ and CS-, whereas continued differential CRs to CS+ relative to CS- are indicative of retrieval 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.

Previous research has associated trait anxiety with physiological responses in threat acquisition (Indovina et al., 2011; Sjouwerman et al., 2017). However, findings on the effects of IU on threat acquisition are largely inconclusive. Though research has demonstrated effects of IU on psychophysiological markers of threat acquisition, where high IU individuals demonstrate heighted startle or SCRs in response to CS+ vs. CS- (Chin et al., 2016; Sjouwerman et al., 2020), other studies have failed to find such effects (Dunsmoor, Campese, et al., 2015; Mertens & Morriss, 2021; Morriss, Christakou, et al., 2016; Morriss, Macdonald, et al., 2016; Morriss & van Reekum, 2019; Wake et al., 2020). In their review of the role of IU in conditioning, Morriss, Zuj, 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 over trait anxiety on psychophysiological responses during acquisition, as this is more likely to increase perceptions of uncertainty (Morriss, Zuj, 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 and to drive effects of IU over trait anxiety in this conditioning phase (Dunsmoor, Campese, et al., 2015; Morriss et al., 2019; Morriss & van Reekum, 2019). In addition, as time throughout extinction goes on, levels of uncertainty are also likely to change (Morriss, Zuj, et al., 2021). It is consequently important to consider extinction in terms of two temporal periods (early and late), as opposed to analysing CRs within 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, Niv, 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 greater differentiated CRs to the CS+ relative to the CS- during early extinction, and continue to do so throughout late extinction,

as indexed by several psychophysiological and neural readout measures, such as greater SCRs, corrugator supercilii activity, pupil dilation, and amygdala activity (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).Therefore, CRs and the subsequent success of extinction are engaged in a differential manner as a function of temporal patterns and individual differences in levels of IU. The effects of IU have also consistently been demonstrated over and above trait anxiety throughout the extinction phase (Dunsmoor, Campese, et al., 2015; Lucas et al., 2018; Morriss, 2019; Morriss, Christakou, et al., 2016; Morriss et al., 2015; Morriss, Wake, et al., 2021; Morriss & van Reekum, 2019). However, it is important to note that though a vast number of studies reported this overall pattern of results, these findings are not always replicated (Kanen et al., 2021; Morriss, Biagi, et al., 2020; Morriss, Wake, et al., 2020; Wake et al., 2020).

Furthermore, despite advancements in understanding the involvement of IU in conditioning paradigms, the slower temporal resolution of SCRs (which are the predominant index of IU in conditioning literature) can result in convolved responses, particularly in designs with short inter-stimulus intervals (Ojala & Bach, 2020). Skin conductance responses can also be generated by numerous internal or external processes, that are not specific to particular cognitive processes (Dawson et al., 2007). 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 IU may modulate threat acquisition and extinction.

### 1.2. Eye-Tracking, Attentional Bias, 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 (Holmqvist & Andersson, 2017; Klein & Ettinger, 2019). As a result, the fovea is typically repositioned to direct gaze towards stimuli that are being thought of or processed in the moment. Attention is therefore thought to be intimately related to volitional movements of the head or eyes (Rayner, 2009; Ward, 2020). This makes eye-tracking a reliable and ecologically valid method for directly observing and continuously assessing the allocation of visual attention and 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, the length of time in which the eye pauses, and the angular distance traversed between successive fixations, which are termed the number of fixations, fixation duration, and saccade amplitude, respectively (Hannula, 2010; Holmqvist & Andersson, 2017). Fixations enable extraction of detailed information about an object, with longer fixation durations indicating more extensive or elaborate processing. Saccades in turn allow exploration of the environment by rapidly redirecting the line of sight to a new object of interest, and larger saccade amplitudes therefore indicate shifts between points of interest (Holmqvist & Andersson, 2017; Klein & Ettinger, 2019). Attentional bias,

or hyper-vigilance, is therefore a state of heightened awareness thought to be indexed by global eye-movements such as fewer but longer fixations with decreased saccade amplitude. Contrastingly, 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 with increased saccade amplitude (Horley et al., 2003).

A large body of research has demonstrated that animals as well as humans exhibit sustained vigilance when faced with threat, where increases in attentional bias reflect information-gathering behaviour (Davis & Whalen, 2001; Grupe & Nitschke, 2011; Hogarth et al., 2008; Whalen, 2007), which has also been consistently demonstrated in anxious populations (Armstrong & Olatunji, 2012; Bar-Haim et al., 2007; Cisler & Koster, 2010). The measurement of attentional bias through eye-tracking has typically been combined with spatial attention paradigms, such as the dot probe task (Bar-Haim et al., 2007; Mogg & Bradley, 1998), visual search task (Cisler et al., 2009; Miltner et al., 2004; Ohman et al 2001; Rinck et al., 2003), spatial cueing task (Bar-Haim et al., 2007; Cisler et al., 2009), and modified Stroop task (Bar-Haim et al., 2007; Mogg & Bradley, 1998). Each of these studies have reported attentional bias towards threat relative to neutral cues, which is characterised by faster or shorter reaction times depending on the chosen paradigm (see Cisler & Koster, 2010 or Koster & Bogaerde, 2018 for review). However, the conclusions drawn from this research are based on reaction times, and though these studies show correspondence between manual responses and eye-movements, reaction-time-based attention indices capture only indirect effects of attention and demonstrate poorer reliability than eye-tracking alone (Rodebaugh et al., 2016; Waechter et al., 2014). The use of eye-tracking as a measure in itself may therefore provide more sensitive indices of attentional bias. Furthermore, as attending to threat signals is adaptive for animals and humans, using gaze to gather information about possible threats has potential benefits. Considering meta-analytic evidence from eye-tracking studies indicating that anxiety is characterised by attentional bias to threat (Armstrong & Olatunji, 2012; Bar-Haim et al., 2007; Cisler & Koster, 2010), and the notion that threat acquisition and extinction are thought to respectively represent the development and treatment of fear and anxiety (Craske et al., 2014; Mineka & Zinbarg, 2006), it is surprising that eye-movements have not been investigated in the context of conditioning until very recently.

Recently, Xia et al. (2020) explored the effects of conditioning using eye-tracking technology. As this is the first study to our knowledge to integrate this methodology into conditioning paradigms, it will be considered in more detail. The researchers investigated how a visual search metric - scanpath length (the sum of all saccade amplitudes in a scanpath, Holmqvist & Andersson, 2017) - varied throughout threat acquisition (Experiments 1-3), and in threat extinction (Experiment 3). In the acquisition phase, participants were presented with full-screen monochrome (Experiments 1 and 2) or patterned stimuli (Experiment 3) (CSs), which were reinforced with electrical pulses (US) using a 50% reinforcement rate in acquisition. Consistent conditioning effects were found throughout acquisition across all three experiments, whereby scanpath length was significantly shorter in

response to CS+ vs CS-, and these effects then extinguished in Experiment 3, as scanpath length no longer differed between CS+ and CS-. Xia et al. also sought to understand the mechanism of eye movements underlying scanpath length. They found that shorter scanpath length in response to the CS+ was characterised by longer fixation durations, whereas longer scanpath length in response to the CS- was characterised by shorter fixation duration, increased scanning, and scanning of a wider screen area. These results align with attentional bias literature and demonstrated for the first time that eye-tracking can be utilised to quantify conditioning, as there were more hyper-vigilant behaviours (shorter scanpath length/ longer fixation duration) towards threat relative to neutral stimuli throughout acquisition, which then dissipated throughout extinction.

Whilst this was an important methodological contribution, particularly on the use of eye-tracking in acquisition, there is limited evidence on the effects of extinction on eye-movements. As this is the only study to our knowledge to investigate eye-tracking and conditioning, this work requires replication. Furthermore, the extinction phase was treated as a whole, and as described above, the inherent shifts in uncertainty throughout extinction can affect the adjustment of associations (Morriss, Zuj, et al., 2021). There is likewise a substantial gap in our knowledge on the relationship between individual differences and attentional bias behaviours, which this initial study on threat acquisition and extinction did not address. Variations in attentional bias behaviours in conditioning paradigms, captured using eye-tracking technology, therefore merit further research, particularly as a function of individual differences in IU, as this factor has been demonstrated to impair threat extinction.

### 1.3. Eye-Tracking, Attentional Bias, and Intolerance of Uncertainty

Findings from research on attentional bias in anxiety evaluated above may therefore enable predictions about how eye-movements may differ as a function of IU. In the context of anxiety, hypervigilance is defined as a state of heightened perceptual sensitivity to potentially threatening stimuli (Beck & Clark, 1997). If we were to apply this definition to IU, which is characterised by fear of the unknown (Carleton, 2016a, 2016b), we can theorise that in individuals with high levels of IU, perceptual sensitivity would be heightened to potentially *uncertain* stimuli. Indeed, there is experimental evidence demonstrating that higher levels of IU are associated with facilitated engagement towards stimuli that are perceived to be uncertain, e.g. ambiguous words such as 'maybe' (Fergus et al., 2013). Initial investigations into the relationship between IU and attentional biases to uncertain information have demonstrated effects of higher levels of self-reported IU over trait anxiety on attentional bias behaviours towards uncertain information (Morriss et al., 2018; Morriss & McSorley, 2019). These attentionally biased information-seeking behaviours likely serve as coping responses to mitigate or eliminate unknowns (Carleton, 2016a). Attentional bias is therefore likely to differ as a function of IU, particularly in inherently uncertain situations such as partially reinforced stimuli in acquisition, or throughout the extinction phase of conditioning paradigms. Consequently,

high IU individuals may display more hyper-vigilant information-gathering behaviours, such as fewer but longer fixations with decreased saccade amplitude, compared with low IU individuals, in response to uncertainty.

However, when faced with uncertainty, individuals with high levels of IU adopt maladaptive behaviours such as disproportionate information-seeking *or* avoidance (Birrell et al., 2011; Buhr & Dugas, 2002; Carleton et al., 2016; Dugas et al., 2004; Ladouceur et al., 1997; Rosen et al., 2010). It is therefore important to consider that high levels of IU may modulate either hyper-vigilant *or* hyper-scanning behaviours. High IU individuals may consequently demonstrate excessive search for threat cues, reflecting a state of hyper-vigilance, or may engage in avoidance and hyper-scanning behaviours. The use of eye-tracking methodology may therefore prove particularly beneficial in differentiating such behaviours and delineating the nature of attentional bias in IU.

### 1.4. The Present Study

This project involved a secondary analysis of a previously published dataset (Morriss, Wake, 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. We investigated the effects of IU on hyper-vigilant and hyper-scanning behaviours using a direct measure of visual information seeking and global attention: eye-tracking. As there is inherent uncertainty in conditioning, specifically in threat extinction (Levy & Schiller, 2021), we measured these behaviours in the context of a conditioning paradigm. As in previous work (Morriss et al., 2019; Morriss & van Reekum, 2019), we utilised monochromatic coloured squares as CSs, which were reinforced with an unpleasant sound (US) 50% of the time in acquisition. This was followed by an extinction phase, in which the CSs were presented alone. Whilst our predictions were informed by Xia et al.'s (2020) findings, we utilised differing eye-movement variables to characterise attentional bias. Whereas Xia et al. utilised scanpath length, we extracted comparable indices of global attention (fixation count, fixation duration, and saccade amplitude), as these would provide richer information on individual differences in hyper-vigilant and hyper-scanning behaviours. We additionally chose to use these indices because this is the first study to our knowledge to investigate the effects of individual differences, specifically in IU, on threat acquisition and extinction as indexed by eye-movements, and we sought to initially establish how these may differ as a function of IU on a global level.

The proposed analysis aimed to (1) replicate existing but separate findings on eye-tracking in conditioning (Xia et al., 2020) and on the effects of IU in threat extinction (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), 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 CRs compared with low IU individuals.

Furthermore, based on previous research on eye-tracking in conditioning (Xia et al., 2020), as well as existing research on IU in extinction (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) 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 CRs. 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 an IU-Stimulus interaction was estimated using G*Power (Faul et al., 2009) using a repeated-measures within-between interaction design. The following parameters were entered: $f = .26$, $\alpha = .05$, $\beta = .95$, 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, Wake, 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, $\leq 65$) 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 (HD 206, Sennheiser, Wendemark-Wenneboste, Germany).

### 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 held against the headphones prior to each session.

### 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, Zuj, et al., 2021). 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 (see Fig. 1). There were no breaks between acquisition and extinction phases.

**Figure 1**

*Image Depicting (a.) Experimental Conditions and Procedure (b.) Acquisition (50% Reinforced) and Extinction Phases, as well as (c.) Example of Two Consecutive Trials.*
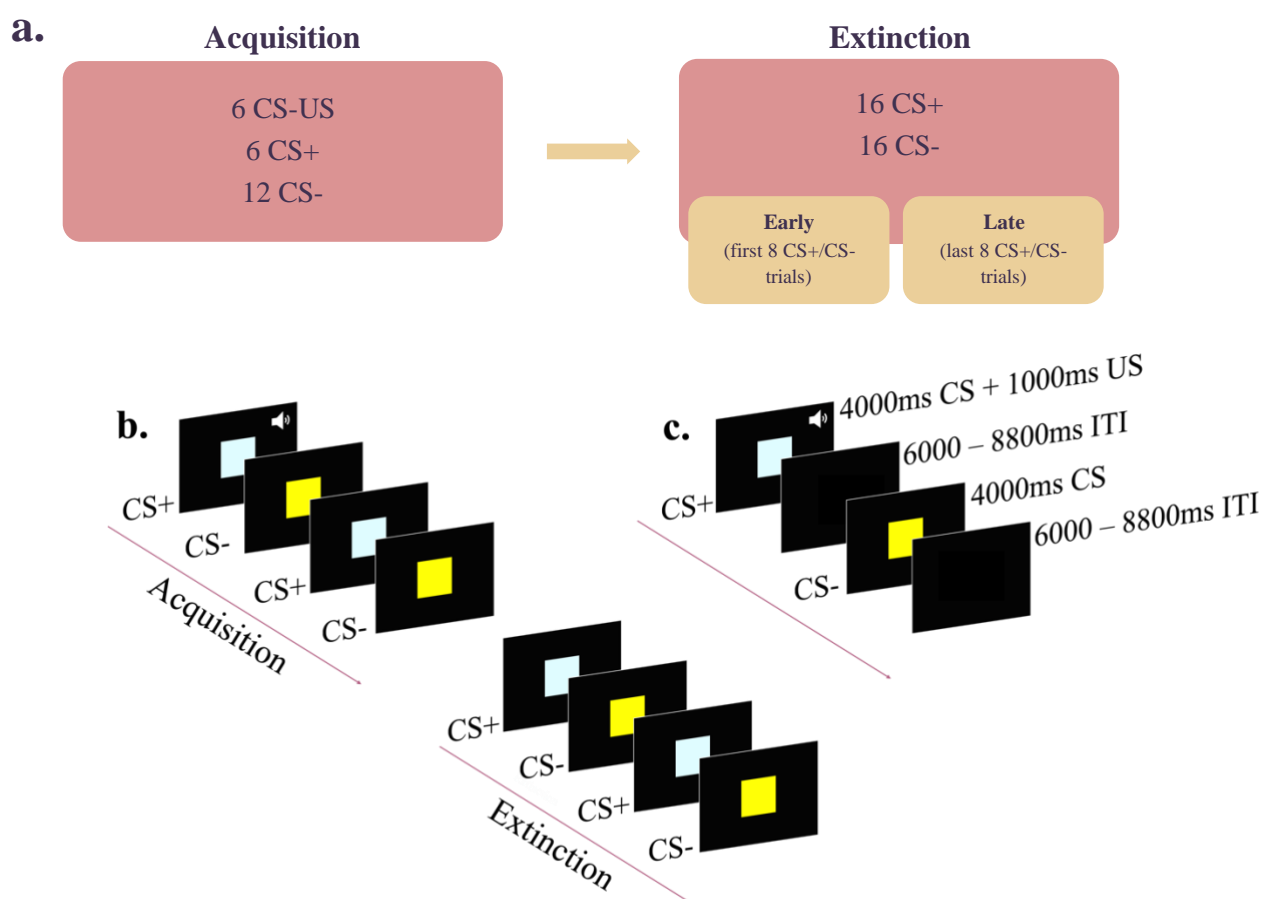


*Note*. CS+ = reinforced conditioned stimulus; CS- = unreinforced conditioned stimulus; US = unconditioned stimulus; ITI = inter-trial-interval. The US co-terminated with the CS+.

### 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 (EyeLink II Manual, SR Research). 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 (°/ms)).

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). 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. Eye-movements 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.

Distributions of oculomotor measures 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, unless otherwise stated. Effect sizes were interpreted based on Cohen's (1988) conventions, whereby $\eta^2_p$ = .01, .06, and .14 indicated small, medium, and large effects, respectively.

### 3.1. Questionnaires

The reliability of IUS and STICSA questionnaires was satisfactory, with respective internal consistencies of α = .95 and α = .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.*
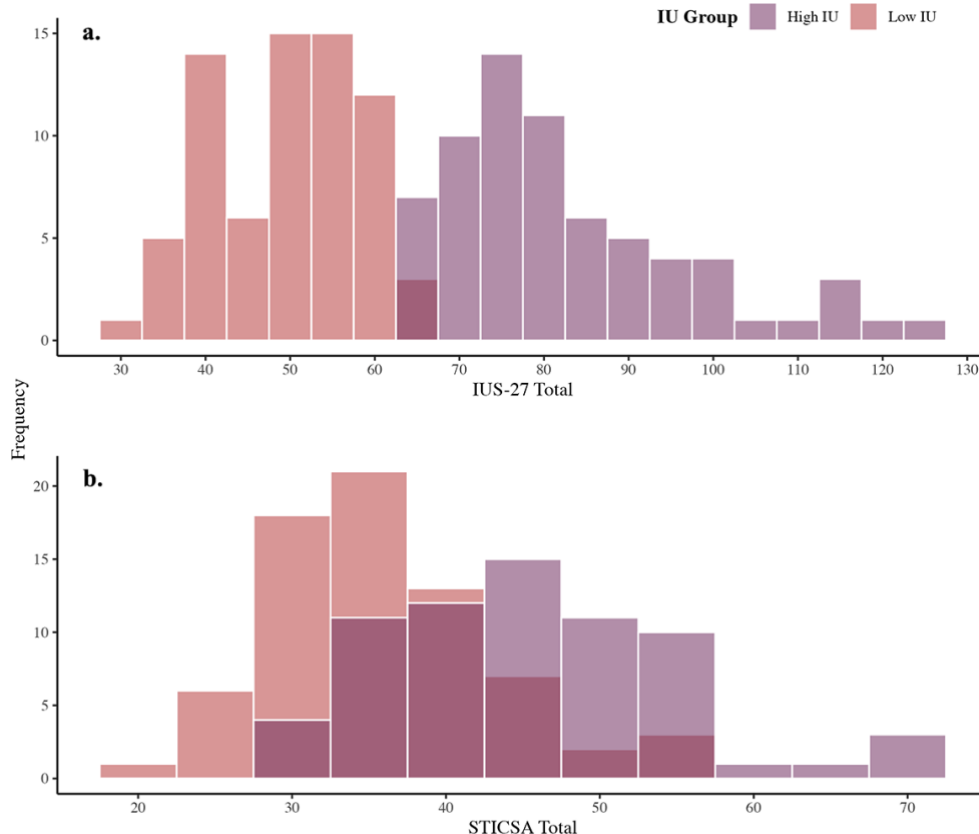


*Note*. Darker shade indicates overlap of high and low IU group scores.

### 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, 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. The R package *rstatix* was used to compute ANOVAs (version 0.7.0, Kassambara, 2021). In the case of significant interactions and in line with a-priori hypotheses, simple main effects were then conducted to better understand the pattern of results, while controlling for the likelihood of Type I errors using the Bonferroni correction.

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

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

### 3.2.1. Acquisition

As demonstrated by the descriptive statistics in Table 1, fixation counts were lower in response to the CS+ than CS- [Stimulus: $F(1,137) = 11.44$, $p < .001$, $\eta^2_p = .08$]. The high IU group had higher fixation counts than the low IU group [IU: $F(1,137) = 4.81$, $p = .030$, $\eta^2_p = .03$]. The IU-Stimulus interaction was not significant [$F(1,137) = 0.26$, $p = .613$, $\eta^2_p < .01$].

Furthermore, whilst fixations were longer in response to the CS+ relative to the CS-, this effect was not significant [Stimulus: $F(1,137) = 2.92$, $p = .090$, $\eta^2_p = .02$]. Fixation durations throughout acquisition differed significantly between IU groups [IU: $F(1,137) = 3.91$, $p = .050$, $\eta^2_p = .03$], with shorter fixation durations in the high IU group than in the low IU group (see Table 1). The IU-Stimulus interaction was not significant [$F(1,137) = 1.27$, $p = .261$, $\eta^2_p = .01$].

Whilst saccade amplitudes were smaller in response to the CS+ vs. CS-, and in the low vs. high IU group, there was no significant difference in fixation count detected across both levels of stimulus [Stimulus: $F(1,136) = 0.95$, $p = .332$, $\eta^2_p = .01$], between IU groups [IU: $F(1,136) = 2.98$, $p = .086$, $\eta^2_p = .02$], and no significant IU-Stimulus interaction [$F(1,136) = 0.38$, $p = .539$, $\eta^2_p < .01$] on saccade amplitude throughout acquisition.
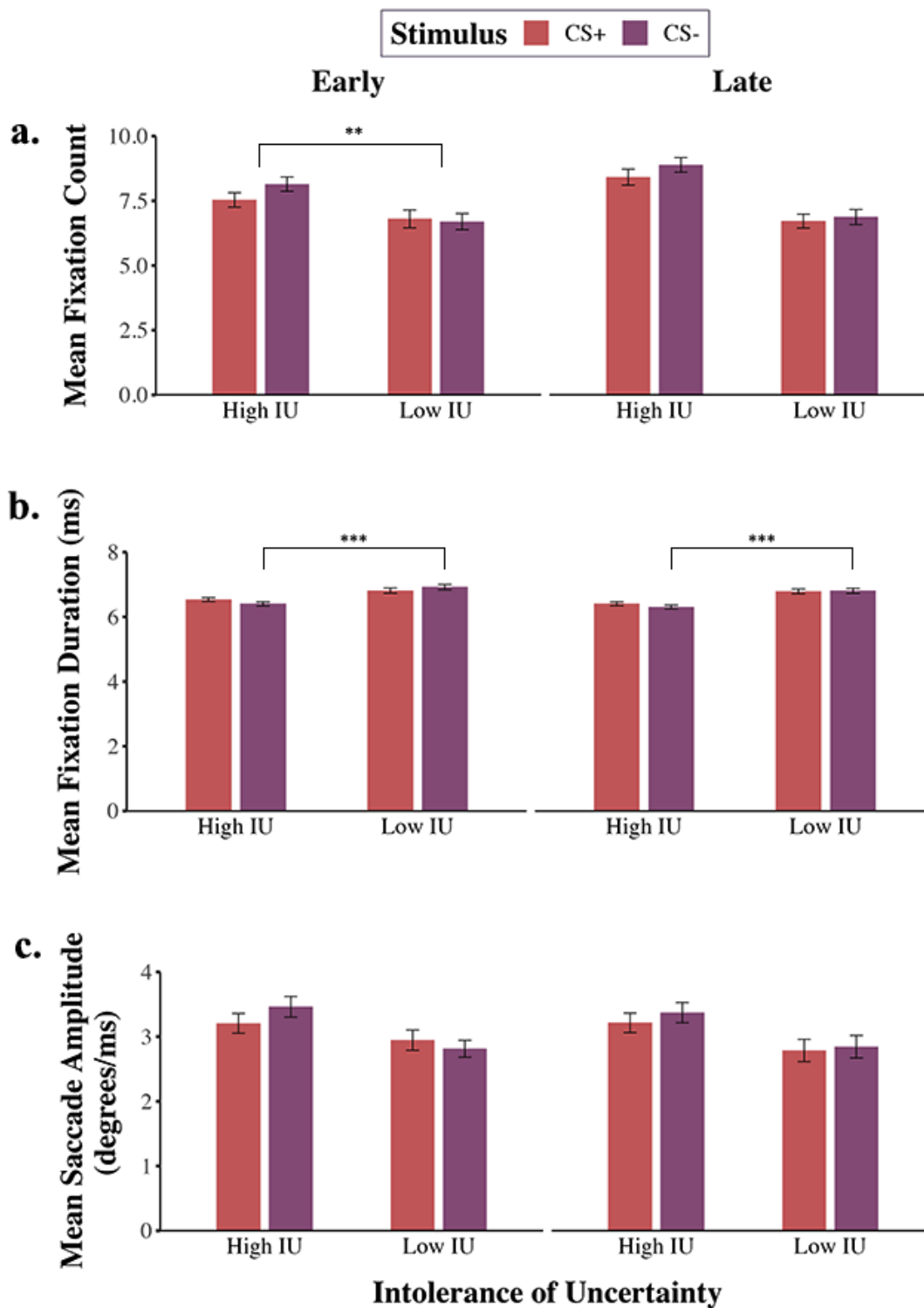
### 3.2.2. Extinction

As reported in Table 1, there were overall fewer fixations to the CS+ than the CS- in extinction, and the ANOVA revealed that this difference was statistically significant [Stimulus: $F(1,137) = 4.16$, $p = .043$, $\eta^2_p = .03$]. Low IU individuals had significantly fewer fixations than high IU individuals [IU: $F(1,137) = 7.67$, $p = .006$, $\eta^2_p = .05$], and there were overall fewer fixations in early versus late extinction [Time: $F(1,137) = 5.73$, $p = .018$, $\eta^2_p = .05$]. Furthermore, there was a statistically significant interaction between IU and Time [$F(1,137) = 4.57$, $p = .034$, $\eta^2_p = .03$] on fixation count in extinction. To break down this interaction, simple main effects compared each level of time across IU groups. These revealed that there was a statistically significant difference in fixation count between IU groups specifically during early extinction [$F(1,137) = 11.41$, $p = .006$, $\eta^2_p = .08$], whereby high IU individuals demonstrated a greater fixation count than low IU individuals (see Table 1 and Figure 3a). There was a similar pattern in late extinction, as demonstrated in Figure 3a, but this was not statistically significant [$F(1,137) = 3.63$, $p = .354$, $\eta^2_p = .03$]. The interaction therefore appeared to be driven by differential fixation counts between IU groups in early extinction only. All other interactions were not statistically significant: IU-Stimulus [$F(1,137) = 3.46$, $p = .065$, $\eta^2_p = .03$], Stimulus-Time [$F(1,137) = 0.06$, $p = .806$, $\eta^2_p < .01$], IU-Stimulus-Time [$F(1,137) = 0.60$, $p = .440$, $\eta^2_p < .01$].

Furthermore, fixation durations did not differ depending on stimulus in extinction, [Stimulus: $F(1, 137) = 0.51$, $p = .477$, $\eta^2_p < .01$]. As demonstrated by Table 1 and Figure 3b, the high IU group demonstrated shorter fixations than the low IU group, and this was statistically significant [IU: $F(1,137) = 11.21$, $p < .001$, $\eta^2_p = .08$]. There was a significant IU-Stimulus interaction [$F(1,137) = 5.82$, $p = .018$, $\eta^2_p = .04$] on fixation duration in extinction. Simple main effects revealed that differences in fixation duration between IU groups in response to the CS- were statistically significant [$F(1,137) = 14.43$, $p = .001$, $\eta^2_p = .10$], with high IU individuals demonstrating shorter fixation

**Figure 3**

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



*Note.* Fixation duration data presented here are log-transformed. Error bars denote standard error of the mean.

$^{*}$ $p \leq .05$, $^{**}$ $p \leq .01$, $^{***}$ $p \leq .001$. All $p$-values depicted here were Bonferroni-adjusted.

durations throughout extinction than low IU individuals, as demonstrated by Table 1 and Figure 3b. This pattern was similar in response to the CS+ as high IU individuals also demonstrated shorter fixation durations than low IU individuals, however this was not statistically significant [$F(1,137) = 6.70$, $p = .066$, $\eta^2_p = .05$]. In addition, fixation durations differed throughout the extinction phase, as there were overall significantly longer fixations in early vs. late extinction [Time: $F(1,137) = 4.35$, $p = .039$, $\eta^2_p = .03$]. There were no further significant interaction effects on fixation duration throughout extinction: IU-Time [$F(1,137) = 0.24$, $p = .624$, $\eta^2_p < .01$], Stimulus-Time [$F(1,137) = 0.16$, $p = .680$, $\eta^2_p < .01$], IU-Stimulus-Time [$F(1,137) = 0.95$, $p = .333$, $\eta^2_p = .01$]).

Saccade amplitude was not associated with stimulus throughout extinction [Stimulus: $F(1,134) = 0.74$, $p = .391$, $\eta^2_p < .01$], and did not differ depending on levels of IU [IU: $F(1,134) = 3.17$, $p = .077$, $\eta^2_p = .02$], or as a function of time [Time: $F(1,134) = 0.28$, $p = .601$, $\eta^2_p < .01$]. From Figure 3c, it appears that the effects of stimulus on saccade amplitude in early extinction differed depending on IU group, with high IU individuals demonstrating smaller saccade amplitude towards the CS+ than the CS-, and low IU individuals demonstrating greater saccade amplitude towards the CS+ vs. the CS-. However, the ANOVA failed to support this interpretation, revealing no significant interaction between IU and Stimulus [$F(1, 134) = 1.69$, $p = .196$, $\eta^2_p = .01$], IU-Time [$F(1,134) = 0.13$, $p = .718$, $\eta^2_p < .01$], Stimulus-Time [$F(1,134) = 0.08$, $p = .781$, $\eta^2_p < .01$], or IU-Stimulus-Time [$F(1,134) = .61$, $p = .437$, $\eta^2_p < .01$].

### 3.3. Specificity of IU over Trait Anxiety

To further evaluate 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. 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, with Bonferroni corrections applied for multiple comparisons. Only changes to the observed effects reported above resulting from the inclusion of STICSA as a covariate are reported here.

#### 3.3.1. Acquisition

Despite a non-significant effect of STICSA [$F(1,136) = 0.06$, $p = .808$, $\eta^2_p = < .01$], the ANCOVA revealed that the previously observed main effect of IU on fixation count was no longer significant after controlling for STICSA [$F(1,136) = 3.19$, $p = .076$, $\eta^2_p = .02$]. This demonstrates that there is no specificity of IU over trait anxiety on fixation count in acquisition. All other observed effects remained the same even after controlling for trait anxiety.

Furthermore, though individual levels of trait anxiety were not significantly associated with fixation duration throughout acquisition [STICSA: $F(1,136) = 0.27$, $p = .606$, $\eta^2_p < .01$], the effect of

IU was no longer significant [$F(1,136) = 3.89$, $p = .051$, $\eta^2_p = .03$]. This suggests that the previously observed effect of IU on fixation duration in acquisition was not specific to IU. All other observed effects on fixation duration remained the same.

The ANCOVA further revealed that the effect of STICSA on saccade amplitude in acquisition was not significant [STICSA: $F(1,134) = 0.01$, $p = .935$, $\eta^2_p < .01$], and its inclusion in the analyses did not affect the non-significant main effects or interactions reported above.

### 3.3.2. Extinction

ANCOVA analyses revealed that there was a non-significant association between trait anxiety and fixation count in extinction [STICSA: $F(1,136) = 0.43$, $p = .512$, $\eta^2_p < .01$]. The previously observed effect of IU remained significant even after accounting for trait anxiety [IU: $F(1,136) = 4.36$, $p = .039$, $\eta^2_p = .03$], suggesting specificity of IU over trait anxiety on fixation count in extinction. The previously observed IU-Time interaction was no longer significant after controlling for STICSA [$F(1,136) = 3.49$, $p = .064$, $\eta^2_p = .03$], which demonstrates that this interaction was not specific to IU. Furthermore, after controlling for STICSA, there was a significant IU-Stimulus interaction [$F(1,136) = 4.56$, $p = .035$, $\eta^2_p = .03$], which suggested that high and low IU groups were affected by stimulus presentation differently, after trait anxiety was controlled for. Despite this, simple main effects revealed no significant associations in fixation counts between IU groups at either level of stimulus following corrections for multiple comparisons [CS+: $F(1,136) = 2.17$, $p = .1.00$, $\eta^2_p = .02$; CS-: $F(1,136) = 6.66$, $p = .110$, $\eta^2_p = .05$]. However, these simple effects did yield small to medium effect sizes.

The covariate, trait anxiety, was not significantly related to fixation duration in extinction [STICSA: $F(1,136) = 0.01$, $p = .972$, $\eta^2_p < .01$]. However, there continued to be a significant main effect of IU [$F(1,136) = 8.37$, $p = .004$, $\eta^2_p = .06$] after controlling for STICSA, which suggests that this effect is specific to IU over trait anxiety. The significance and pattern of all other main effects and interactions reported above remained the same after controlling for trait anxiety.

The ANCOVA analyses further demonstrated a similar pattern throughout extinction on saccade amplitude as the observed mixed ANOVA effects. There were no significant main effects or interactions even after controlling for trait anxiety, which was also not significantly associated with saccade amplitude throughout extinction [STICSA: $F(1,133) = 1.13$, $p = .289$, $\eta^2_p = .01$].

# 4. DISCUSSION

## 4.1. Summary of Findings

Here we utilised eye-tracking methodology to examine the effect of individual differences in IU on threat acquisition and extinction, as indexed by global measures of attention: fixation count, fixation duration, and saccade amplitude. Throughout acquisition, the data demonstrated a pattern of results conducive with CRs in both IU groups, with fewer but longer fixations and decreased saccade amplitudes in response to the CS+ than CS-. This was consistent with our hypotheses and indicated successful formation of a threat association, as responses reflected more hyper-vigilant behaviours towards the CS+ than the CS-. However, analyses revealed that this effect in acquisition was only significant in terms of fixation count. The data likewise demonstrated some effects of IU in acquisition, however, ANCOVA analyses further revealed that the effects of IU on fixation count and fixation duration were no longer significant after controlling for trait anxiety, demonstrating that the observed effects were not IU-specific in the acquisition phase. However, trait anxiety was not significantly related to any of the outcome variables.

Findings in extinction revealed an interesting pattern of results. In early extinction, the high IU group demonstrated continued CRs, with a lower number of longer fixations and decreased saccade amplitude in response to the CS+ relative to CS-. This was as predicted. However, contrary to hypotheses, the low IU group appeared to have extinguished CRs, as these individuals did not demonstrate differentiated responses to the CS+ vs CS- in early extinction. In late extinction, high IU individuals continued to demonstrate CRs to the threat vs. safety cues, as indexed by fewer fixations, longer fixation duration, and decreased saccade amplitude to the CS+ relative to CS-. This indicated unsuccessful extinction retrieval and was in line with our hypotheses. Contrastingly, the descriptive data indicated that the low IU group spontaneously recovered CRs in late extinction, as they demonstrated fewer fixations and decreased saccade amplitude towards the CS+ than to the CS-. Overall, effects of IU and Time in extinction suggest that eye-movements, specifically fixations, differ depending on temporal shifts and individual differences in levels of IU throughout this phase in conditioning. ANCOVA analyses further demonstrated that the effects of IU on fixation count and fixation duration were specific to IU, and that trait anxiety was not significantly related to any of the outcome variables throughout extinction.

## 4.2. Application of Findings to Theory and Literature

We were able to replicate previous work demonstrating that eye-tracking is a viable index of conditioning (Xia et al., 2020), as participants overall demonstrated a lower number of longer fixations with decreased scanning to threat relative to safety stimuli throughout acquisition, which were indicative of hyper-vigilant behaviour. This was comparable to Xia et al.'s findings of shorter

scanpath length in response to CS+ than CS-, which was characterised by longer fixations towards the threat and shorter fixations with increased scanning behaviour in response to the safety stimuli. Our results are also in line with wider attentional bias literature that has demonstrated hyper-vigilance towards threat (Armstrong & Olatunji, 2012; Bar-Haim et al., 2007; Cisler & Koster, 2010). Whilst these effects were significant for fixation count only, the effect size of stimulus on each of the three eye-movement variables throughout acquisition (fixation count: $\eta^2_p = .08$; fixation duration: $\eta^2_p = .02$; saccade amplitude: $\eta^2_p = .01$) was comparable to that of Xia et al.'s observed small-medium effect on scanpath length in acquisition (Experiment 3: Hedge's $g = .42$).[1] Furthermore, the effects of stimulus on fixation count continued to be significant throughout extinction, and from our inferential analyses we cannot conclude that fixation duration and saccade amplitude responses to CS+ vs CS- extinguished, as there were no significant effects of stimulus on these two variables in acquisition. However, we did not expect for individuals in the high IU group to extinguish CRs. The data also indicated that the low IU group successfully extinguished CRs in early extinction, as they did not appear to differentiate between threat and safety stimuli across all three eye-tracking measures. Their behaviour in late extinction was contrary to hypotheses and will be discussed in further detail below. Interestingly, Xia et al.'s experiment (Experiment 3) consisted of 40 trials throughout extinction, whilst ours included 32 trials. In addition, whilst there were no breaks between acquisition and extinction in our experiment, their design included a self-paced break in between acquisition and extinction phases, which may have facilitated extinction of CRs in their sample, as it may have made changes in contingencies more obvious (Morriss, Zuj, et al., 2021). In addition, comparison of the resulting effect sizes in our data from extinction to acquisition tentatively indicated overall successful extinction of CRs, as these decreased from acquisition into extinction (fixation count: acquisition $\eta^2_p = .08$, extinction $\eta^2_p = .03$; fixation duration: acquisition $\eta^2_p = .02$, extinction $\eta^2_p < .01$; saccade amplitude: acquisition $\eta^2_p = .01$, extinction $\eta^2_p < .01$). Extinction effect sizes were likewise comparable to that of Xia et al. (Experiment 3: Hedge's $g < .01$). Our data therefore tentatively indicate successful extinction of CRs, in line with previous research incorporating eye-tracking technology in conditioning paradigms (Xia et al, 2020).

Our findings also replicated previous work demonstrating that high levels of self-reported IU are associated with impaired threat extinction (Bauer et al., 2020; Morriss, 2019; Morriss, Christakou, et al., 2016; Morriss et al., 2015, 2019; Morriss, Macdonald, et al., 2016; Morriss, Wake, et al., 2021; Morriss & van Reekum, 2019; Wake et al., 2021). This was indexed by continued CRs in high IU individuals throughout both phases of extinction, which was characterised by fewer and shorter fixations and less scanning behaviours in response to threat versus safe stimuli, though notably only effects on fixation count continued to be significant throughout extinction. Such hyper-vigilant behaviours in response to uncertain threat also confirm those of previous investigations into the role

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[1] *Note*. Hedge's $g$ effect sizes interpreted as follows: small: $g = 0.2$; medium: $g = 0.5$; large: $g = 0.8$.

of IU in attentional bias to uncertain affective information (Morriss et al., 2018; Morriss & McSorley, 2019), where high IU individuals' eyes were drawn more towards stimuli with increased perceived threat uncertainty, indicating information-gathering rather than avoidance behaviours towards uncertain threat. This further aligns with conceptual understanding of IU, whereby attentionally-biased information-seeking behaviours towards uncertain threat, as demonstrated by our data, likely serve as elimination of unknowns in high IU individuals (Carleton, 2016a). Surprisingly, individuals with lower levels of IU initially extinguished CRs and demonstrated extinction retrieval in early extinction. They then appeared to spontaneously recover, as their average eye-movements indicated that in late extinction they had fewer fixations and decreased saccade amplitude towards the CS+ vs. CS-. These findings are not in line with the majority of previous research on IU in conditioning (Bauer et al., 2020; Morriss, 2019; Morriss, Christakou, et al., 2016; Morriss et al., 2015, 2019; Morriss, Macdonald, et al., 2016; Morriss, Wake, et al., 2021; Morriss & van Reekum, 2019; Wake et al., 2021). However, it is possible to place these unexpected effects in the low IU group in wider attentional bias literature. Notably, our findings are based on the assumption that eye-movements and attention are intricately linked (Carter & Luke, 2020; Felmingham et al., 2011; Rayner, 2009; Ward, 2020). Whilst this is a reasonable assumption, the external environment can also be attended to by means of covert attention, whereby information outside the fovea is processed and held in mind (Henderson et al., 1989). It is likewise possible that, despite attention to a specific object or stimulus, this attention may not capture interest, but rather boredom or confusion (Holmqvist & Andersson, 2017). Low IU individuals may therefore have quickly learned the updated contingencies, which would explain their extinguished CRs in early extinction. It is possible that these individuals then disengaged from the task, and attentional bias behaviours with fewer eye movements in late extinction indicated boredom, rather than spontaneous recovery. Our measures did not quantify spatial aspects that could perhaps measure whether participant fixations were on stimuli or outside of stimulus areas, or obtain engagement ratings from participants. It is therefore important to interpret these findings with caution.

Furthermore, we found that despite hyper-vigilant behaviours towards threat vs safety stimuli, when compared with low IU, higher levels of IU were overall related to more hyper-scanning and avoidance behaviours, as indexed by a larger number of short fixations with increased saccade amplitude. This pattern of behaviour is thought to represent avoidance (Horley et al., 2003) and has implications for our understanding of IU. Increasingly robust evidence suggests that IU comprises of two dimensions: *prospective* IU, and *inhibitory* IU, which are thought to reflect a desire for predictability and uncertainty paralysis, respectively (Birrell et al., 2011; Carleton, 2016a; McEvoy & Mahoney, 2011). These dimensions are likewise thought to respectively represent approach and avoidance approaches to uncertainty (Birrell et al., 2011), and are often associated with opposing psychophysiological responses (Jackson et al., 2016; Nelson et al., 2015). Prospective and inhibitory IU have also been differentially associated with mental health disorders (Berenbaum et al., 2008;

Carleton et al., 2012; Mahoney & McEvoy, 2012; McEvoy & Mahoney, 2011, 2012). It is therefore likely that hyper-vigilance and hyper-scanning behaviours are modulated differently by these two subscales. As we treated IU unimodally in our analyses, we did not investigate any differences between high and low IU groups in terms of prospective or inhibitory IU. It is possible that IU scores in the high IU group were driven by the inhibitory subscale, which is more conducive of hyper-scanning behaviours, whereas low IU may have been characterised by an overall majority of prospective IU. This is not something we explored, and future studies may wish to address these differential behaviours when investigating eye-tracking and IU.

We further found that as in the majority of prior work, the observed effects were specific to IU over trait anxiety in extinction (Dunsmoor, Campese, et al., 2015; Lucas et al., 2018; Morriss, 2019; Morriss, Christakou, et al., 2016; Morriss et al., 2015; Morriss, Wake, et al., 2021; Morriss & van Reekum, 2019). However, despite the use of a 50% reinforcement schedule, which maximises the unpredictability of the CS-US contingency (Morriss, Zuj, et al., 2021), we did not find IU-specific effects on any of our eye-tracking measures throughout acquisition. This is not an entirely surprising finding. Throughout conditioning, uncertainty can be manipulated in several ways (see Morriss et al., 2021 for review). There was no spatial uncertainty in our design, as stimuli were always centred on the screen. Furthermore, though there was an element of increased uncertainty through jittering of ITIs, the CSs were always presented for the same amount of time, and the US always began 3000ms into CS presentation, and co-terminated with the CS. Temporal uncertainty was therefore not maximised, and so contingencies may have been relatively easy to learn throughout acquisition, reducing the overall perceived uncertainty in this phase of conditioning, resulting in non-specific effects of IU.

### 4.3. Strengths, Limitations and Future Directions

A key strength of our study is the potential to apply our findings to treatments, as eye-tracking may likewise be a viable tool for treatment of anxiety and mood-based disorders. Indeed, research on attentional bias modification therapy (Britton et al., 2015; Hakamata et al., 2010), which revolves around retraining attentional bias, has already provided evidence of reduced anxiety symptoms in clinical social anxiety and generalised anxiety disorder populations following treatment (Amir et al., 2009; Lazarov et al., 2017; Price et al., 2016). In addition, IU has been associated with risk for the development of anxiety symptoms and predicts poorer outcomes following treatment (Keefer et al., 2017; Oglesby et al., 2016). There is therefore great potential to utilise eye-tracking to specifically retrain the attentional avoidance behaviours exhibited by individuals with high levels of self-reported IU in a targeted manner, as this is likely the mechanism preventing them from updating threat and safety associations and impairing their treatment outcomes.

Nonetheless, there are several factors which need to be considered in evaluating the findings of the present research. Whilst we accounted for the influence of trait anxiety in our analyses, it

appears that this construct was not related to any of our outcome variables, and there remains a substantial amount of unexplained variance in the observed eye-movements. There are multiple factors that not only affect attentional deployment, such as working memory (Oberauer, 2019), but also those that influence the quality of data recorded using eye-tracking measures, including eye colour, the use of glasses or contact lenses (dark-coloured irises without contact lenses or glasses maximise precision) (Holmqvist & Andersson, 2017), which were not controlled for in our study and may have accounted for some unexplained variance. Whilst the omission of such factors may have improved generalisability of our data, these factors should be considered in future eye-tracking studies investigating individual differences in attentional bias to obtain more precise data.

In addition, it is important to consider biological individual differences such as age, ethnicity and sex in conditioning (Lonsdorf & Merz, 2017). Whilst groups did not differ in terms of age or ethnicity, IU groups were unbalanced according to the sex of participants, with a higher female to male ratio in the high than low IU group (see Appendix B), which was not accounted for in our analyses. This may have affected our findings, as varying levels of hormones are found over the course of the menstrual cycle in females or following use of oral or hormonal contraceptives, all of which have demonstrated critical impacts on cognitive and affective processes (Lisofsky et al., 2016; Sundström Poromaa & Gingnell, 2014; Toffoletto et al., 2014; Warren et al., 2014), and can in turn influence threat acquisition and extinction (Lonsdorf & Merz, 2017).

As this was the first study to our knowledge to combine eye-tracking, conditioning, and IU, we aimed to initially establish how global indices of attention may differ as a function of IU. We utilised eye-movement variables that were suited to this aim, however, we did not find consistent significant effects of conditioning or IU across all three measures. Our work therefore requires replication. To add to this, there was substantial upward drift in the data of many participants. Although calibration and validation procedures were completed at the start of the experiment, we did not build multiple calibrations into the session. In addition, this drift could have resulted from several individual difference factors such as avoidance behaviours, eye colour, or use of glasses (Carter & Luke, 2020; Holmqvist & Andersson, 2017). It is therefore unclear whether this drift resulted from such individual differences or from methodological shortcomings. While this drift was unlikely to affect our measures as they were not spatially dependent, data quality may be improved in future studies through inclusion of fixation points in ITIs and validating calibration throughout the course of the experiment (Carter & Luke, 2020).

With this in mind, future work may additionally turn to refining our findings by investigating more spatially and temporally specific eye-movement variables and focusing on particular stimulus interest areas. These may ascertain the time-course of attentional bias by indexing initial orienting (Mogg & Bradley, 2016), attention maintenance (Koster et al., 2004), and active avoidance behaviours (Pflugshaupt et al., 2007). Future work may additionally wish to consider the prospective and inhibitory IU subscales, as they appear to modulate differing behaviours and cognitions, and in

combination with more spatially and temporally specific eye-tracking variables, they may delineate clearer patterns of hypervigilance and hyper-scanning behaviours in IU. These may be of particular use in formulating tailored attention and exposure-based therapies for disorders with anxiety components (Carleton, 2016a, 2016b; McEvoy & Mahoney, 2012).

# 5. CONCLUSIONS

Here we aimed to determine whether eye-tracking methodology could be utilised to quantify the effects of individual differences in IU on threat acquisition and extinction. We measured global indices of attention: fixation count, fixation duration, and saccade amplitude, which differentiated between hyper-vigilance and hyper-scanning behaviours. We were able to replicate previous work demonstrating that eye-tracking is a viable index of conditioning, as, overall, attention became more focused in response to threat versus safe stimuli throughout acquisition. This was indexed by fewer but longer fixations and less scanning behaviour in response to the threatening than safe stimulus. Furthermore, we were able to replicate previous work demonstrating that high levels of self-reported IU are associated with poorer threat extinction, as high IU individuals continued to demonstrate conditioned responses to threat vs. safe stimuli throughout extinction, indexed by fewer but longer fixations to threatening stimuli, relative to safe. Findings in extinction were specific to IU over trait anxiety.

Finally, when compared with low IU individuals, those with high levels of self-reported IU demonstrated eye-movement patterns that are more reflective of avoidance and hyper-scanning than hyper-vigilance behaviours. This behaviour was characterised by a higher number of shorter fixations with increased saccade amplitude and suggests that high IU individuals may generally engage more in avoidance than disproportionate information-seeking behaviours. Future studies should investigate IU subscales in combination with more spatially and temporally specific eye-movements to improve our understanding of these behaviours. Overall, this study demonstrated for the first time that eye-tracking is a viable psychophysiological measure of the effects of IU in conditioning paradigms, informing models of IU, trait anxiety, attentional bias, as well as attention- and exposure-based therapies.

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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*

Please refer to document on **https://github.com/crodsob/empirical_project/tree/main/appendices** titled 'Appendix I – Tests for Assumptions of Parametric Analyses'.

**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'.